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(21) International Application Number: PCT/US98/08463 (22) International Filing Date: 28 April 1998 (28.04.98) (30) Priority Data: 08/847,616                      28 April 1997 (28.04.97)                      US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US    08/847,616 (CIP) Filed on                                      28 April 1997 (28.04.97) (71) Applicant (for all designated States except US): THE UNIVERSITY OF BRITISH COLUMBIA [CA/CA]; 2255 Westbrook Mall, Vancouver, British Columbia V6T 1Z3 (CA). (72) Inventors; and (75) Inventors/Applicants (for US only): REINER, Peter, B. [US/CA]; 4584 W. 1st Avenue, Vancouver, British Columbia V6R 1H8 (CA). LAM, Fred, Chiu-lai [CA/CA]; 8215 Laurel Street, Vancouver, British Columbia V6P 3V2 (CA). (74) Agents: HANLEY, Elizabeth, A. et al.; Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  Published Without international search report and to be republished upon receipt of that report.														
(54) Title: METHOD AND COMPOSITION FOR MODULATING AMYLOIDOSIS (57) Abstract <p>Methods for modulating amyloid deposition in a subject are described. An effective amount of at least one ATP binding cassette (ABC) transporter blocker is administered to a subject, such that modulation of amyloid deposition occurs. Methods also include administering an effective amount of at least one ABC transporter blocker, or a pharmaceutically acceptable salt thereof, to a subject such that a disease state associated with amyloidosis is treated. Packaged pharmaceutical compositions for treating amyloidosis are described. The package includes a container for holding an effective amount of a pharmaceutical composition and instructions for using the pharmaceutical composition for treatment of amyloidosis. The pharmaceutical composition includes at least one ABC blocker for modulating amyloid deposition in a subject. Methods for identifying agent which modulate amyloid deposition in a subject are also described. An effective amount of at least one ATP binding cassette (ABC) transporter blocker is administered to an organism, such that modulation of amyloid deposition occurs.</p>																
<table border="1"> <caption>APPs (fold increase over control)</caption> <thead> <tr> <th>Treatment</th> <th>APPs (fold increase over control)</th> </tr> </thead> <tbody> <tr> <td>CONTROL</td> <td>1.0</td> </tr> <tr> <td>PMA</td> <td>~1.4*</td> </tr> <tr> <td>RU486</td> <td>~2.2*</td> </tr> <tr> <td>DEX</td> <td>1.0</td> </tr> <tr> <td>PROG</td> <td>~1.6*</td> </tr> <tr> <td>DES</td> <td>~2.4*</td> </tr> </tbody> </table>			Treatment	APPs (fold increase over control)	CONTROL	1.0	PMA	~1.4*	RU486	~2.2*	DEX	1.0	PROG	~1.6*	DES	~2.4*
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## METHOD AND COMPOSITION FOR MODULATING AMYLOIDOSIS

### Background of the Invention

Alzheimer's disease is a common dementing brain disorder of the elderly. The  
5 key features of the disease include progressive memory impairment, loss of language  
and visuospatial skills, and behavior deficits. These changes in cognitive function are  
the result of degeneration of neurons in the cerebral cortex, hippocampus, basal  
forebrain, and other regions of the brain. Neuropathological analyses of postmortem  
Alzheimer's diseased brains consistently reveal the presence of large numbers of  
10 neurofibrillary tangles in degenerated neurons and neuritic plaques in the extracellular  
space and in the walls of the cerebral microvasculature. The neurofibrillary tangles are  
composed of bundles of paired helical filaments containing hyperphosphorylated *tau*  
protein (Lee, V. M and Trojanowski, J. Q, The disordered Cytoskeleton in Alzheimer's  
disease, *Curr. Opin. Neurobiol.* 2:653 (1992)). The neuritic plaques consist of deposits  
15 of proteinaceous material surrounding an amyloid core (Selkoe, D. J., "Normal and  
abnormal biology of the  $\beta$ -amyloid precursor protein", *Annu. Rev. Neurosci.* 17:489-517  
(1994)).

Evidence suggests that deposition of amyloid- $\beta$  peptide ( $A\beta$ ) plays a significant  
role in the etiology of Alzheimer's disease. A portion of this evidence is based upon  
20 studies which have been generated from data with regard to familial Alzheimer's disease.  
To date, this aggressive form of Alzheimer's disease has been shown to be caused by  
missense mutations in (at least) three genes: the amyloid precursor protein (APP) gene  
itself (Goate, A. *et al.*, "Segregation of a missense mutation in the amyloid precursor  
protein gene with familial Alzheimer's disease", *Nature* 349:704-706 (1991) and Mullan,  
25 M. *et al.*, "A pathogenic mutation for probable Alzheimer's disease in the APP gene at  
the N-terminus of  $\beta$ -amyloid", *Nature Genet.* 1:345-347 (1992)), and two genes termed  
presenilins 1 and 2 (Sherrington, R. *et al.*, "Cloning of a gene bearing missense  
mutations in early-onset familial Alzheimer's disease", *Nature* 375:754-760 (1995) and  
Rogaev, E. I. *et al.*, "Familial Alzheimer's disease in kindreds with missense mutations  
30 in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene", *Nature*  
376:775-778 (1995)). The missense mutations in APP are located in the region of the  
protein where proteolytic cleavage normally occurs (see below), and expression of at  
least some of these mutants results in increased production of  $A\beta$  (Citron, M. *et al.*,  
"Mutation of the  $\beta$ -amyloid precursor protein in familial Alzheimer's disease increases  $\beta$   
35 -amyloid production", *Nature* 360:672-674 (1992), Cai, X-D. *et al.*, "Release of excess  
amyloid  $\beta$  protein from a mutant amyloid  $\beta$  protein precursor", *Science* 259:514-516  
(1993) and Reaume, A. G. *et al.*, "Enhanced amyloidogenic processing of the beta-

amyloid precursor protein in gene-targeted mice bearing the Swedish familial Alzheimer's disease mutations and a humanized A $\beta$  sequence", *J. Biol. Chem.* 271:23380-23388 (1996)). Initial analyses of the structure of the presenilins did not suggest whether or not they might alter production of A $\beta$ , however, recent data has

5 indicated that these mutations cause an increase in A $\beta$  secretion (Martins, R. N. *et al.*, "High levels of amyloid- $\beta$  protein from S182 (Glu<sup>246</sup>) familial Alzheimer's cells", 7:217-220 (1995) and Scheuner, D. *et al.*, "Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease", *Nature Medicine* 2:864-870

10 (1996); Borchelt DR, *et al.*, "Familial Alzheimer's disease-linked presenilin 1 variants elevate A $\beta$ 1-42/1-40 ratio *in vitro* and *in vivo*," *Neuron* 17:1005-1013 (1996); Duff *et al.*, "Increased amyloid- $\beta$ 42(43) in brains of mice expressing mutant presenilin 1," *Nature* 383:710-713 (1996); Scheuner *et al.*, "Secreted amyloid  $\beta$ -protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and

15 2 and APP mutations linked to familial Alzheimer's disease," *Nature* 2:864-870 (1996); Citron *et al.*, "Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid  $\beta$ -protein in both transfected cells and transgenic mice," *Nature Medicine* 3:67-72 (1997). Tomita *et al.*, "The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid

20  $\beta$  protein ending at the 42<sup>nd</sup> (or 43<sup>rd</sup>) residue" *Proc Natl Acad Sci USA* 94:2025-2030 (1997)). Thus, increased production of A $\beta$  is associated with Alzheimer's disease. Corroborating evidence has been derived from at least two other sources. The first is that transgenic mice which express altered APP genes exhibit neuritic plaques and age-dependent memory deficits (Games, D. *et al.*, "Alzheimer-type neuropathology in

25 transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein", *Nature* 373:523-525 (1995); Masliah, E. *et al.*, "Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F  $\beta$ -amyloid precursor protein and Alzheimer's disease", *J. Neurosci.* 16:5795-5811 (1996); Hsiao, K. *et al.*, "Correlative memory deficits, A $\beta$  elevation, and amyloid plaques in transgenic mice", *Science* 274:99-103

30 (1996); Sturchler-Pierrat *et al.*, "Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology," *Proc Natl Acad Sci USA* 94:13287-13292 (1997)). The second piece of evidence comes from study of patients suffering from Down's syndrome, who develop amyloid plaques and other symptoms of Alzheimer's disease at an early age (Mann, D. M. A. and M. M. Esiri, "The pattern of

35 acquisition of plaques and tangles in the brains of patients under 50 years of age with Down's syndrome", *J. Neurol. Sci.* 89:169-179 (1989)). Because the APP gene is found on chromosome 21, it has been hypothesized that the increased gene dosage which

results from the extra copy of this chromosome accounts for the early appearance of amyloid plaques (Kang, J. *et al.*, "The precursor protein of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor", *Nature* 325:733-736 (1987); Tanzi, R. E. *et al.*, "Amyloid  $\beta$  protein gene: cDNA, mRNA distribution and genetic linkage near the Alzheimer locus", *Science* 235:880-884 (1987)). Taken together with the evidence derived from cases of familial Alzheimer's disease, the current data suggests that genetic alterations which result in an increase in A $\beta$  production can induce Alzheimer's disease.

At present, less is understood about molecular modifications which are associated with the more common, sporadic form of Alzheimer's disease. It is well-established that allelic variation of apolipoprotein E is highly correlated with expression of Alzheimer's disease (Poirier, J., "Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease", *Trends Neurosci.* 17:525-530 (1994); Roses, A. D. "Perspective on the metabolism of apolipoprotein E and the Alzheimer diseases", *Exp. Neurol.* 132:149-156 (1995)), but the mechanistic implications of this finding remain elusive. As in familial Alzheimer's disease (Suzuki, N. *et al.*, "An increased percentage of long amyloid  $\beta$  protein secreted by familial amyloid  $\beta$  protein precursor ( $\beta$ APP<sub>717</sub>) mutants", *Science* 264:1336-1340 (1994)) and Down's syndrome (Teller, J. K. *et al.*, "Presence of soluble amyloid  $\beta$ -peptide precedes amyloid plaque formation in Down's syndrome", *Nature Medicine* 2:93-95 (1996)), A $\beta$  deposited in sporadic Alzheimer's disease plaques is typically a longer 42 amino acid version, A $\beta$ <sub>42</sub> (Gravina, S. A. *et al.*, "Amyloid beta protein (A beta) in Alzheimer's disease brain: Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42", *J. Biol. Chem.* 270:7013-7016 (1995)). The suggestion that A $\beta$ <sub>42</sub> is particularly pathogenic is consistent with data which demonstrate that this isoform is more lipophilic, aggregates more easily, and is more neurotoxic than its 40 amino acid cousin A $\beta$ <sub>40</sub> (Yankner, B. A., "Mechanisms of neuronal degeneration in Alzheimer's disease", *Neuron* 16:921-932 (1996)). The A $\beta$ <sub>42</sub> phenotypic similarity gives support to the hypothesis that sporadic Alzheimer's disease is also due to increased production of A $\beta$ . What remains unclear is what the nature of the change is in sporadic Alzheimer's diseased brains which lead to increased production of A $\beta$ . However, because A $\beta$  deposition is an early and invariant event in Alzheimer's disease, it is believed that treatment which reduces production of A $\beta$  will be useful in the treatment of this disease.

In addition to Alzheimer's disease, amyloidosis is also implicated in the pathophysiology of both stroke and head trauma. It is well established that neuronal trauma initiated either by cerebral ischemia such as that seen in stroke and head trauma all increase expression of APP and production of A $\beta$  (Siman *et al.*, "Expression of  $\beta$ -amyloid precursor protein in reactive astrocytes following neuronal damage." *Neuron*

3:275-285 (1989).; Abe et al., "Selective induction of kunitz-type protease inhibitor domain-containing amyloid precursor protein mRNA after persistent focal ischemia in rat cerebral cortex." *Neurosci Lett* 125:172-174 (1991).; Roberts et al., " $\beta$ -A4 amyloid protein deposition in brain after head trauma." *Lancet* 338:1422-1423 (1991).;

5 Gentleman et al., " $\beta$ -Amyloid precursor protein ( $\beta$ APP) as a marker for axonal injury after head injury." *Neurosci Lett* 160:139-144 (1993); Yokota et al., "Cytotoxic fragment of amyloid precursor protein accumulates in hippocampus after global forebrain ischemia," *J Cereb Blood Flow Metab* 16:1219-1223 (1996)). Indeed, the syndrome of *dementia pugilistica* which had been distinguished from Alzheimer's

10 disease because of the absence of congophilic plaques (Corsellis et al., "The aftermath of boxing." *Psychological Med* 3:270-303 (1973)) has been shown to be characterized by large numbers of A $\beta$  containing diffuse plaques (Roberts et al., "The occult aftermath of boxing," *J Neurol Neurosurg Psychiatry* 53:373-378 (1990)). Moreover, cerebral amyloid angiopathy is a common feature of the brains of stroke patients with symptoms

15 of dementia, focal neurological syndromes, or other signs of brain damage (Corio and Rubio, "Cerebral amyloid angiopathies", *Neuropath Appl Neurobiol* 22:216-227 (1996)). Taken together, these data suggest that production and deposition of A $\beta$  may contribute to the pathology of Alzheimer's disease, head injury, and stroke.

A large body of data has accumulated regarding the production and deposition

20 of both APP and A $\beta$ . APP is an ubiquitous transmembrane glycoprotein (Selkoe, D. J., "Normal and abnormal biology of the  $\beta$ -amyloid precursor protein", *Annu. Rev. Neurosci.* 17:489-517 (1994)). Three major isoforms of APP are produced by alternative splicing: 751 and 770 amino acid isoforms contain a Kunitz protease inhibitor domain and are expressed both in neuronal and non-neuronal cells, while a 695

25 amino acid isoform lacks this domain and is expressed at high levels in neurons. Mature APP is turned over rapidly, with a half life of ~20-30 minutes. Embedded within the protein is a sequence of 39-43 amino acids which corresponds to the A $\beta$  peptide.

Proteolytic processing of APP yields peptide fragments of varying size. An extensively studied degradative pathway is one in which the APP molecule is cleaved

30 within the A $\beta$  sequence (between residues 16 and 17) by a yet-to-be identified enzyme termed  $\alpha$ -secretase. The resultant ~110-125 kDa soluble extracellular derivative termed APP<sub>s</sub> is rapidly released into the extracellular medium of cultured cells (Weidemann, A. et al., "Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 amyloid protein", *Cell* 57:115-126 (1989); Esch, F. S. et al., "Cleavage of amyloid  $\beta$  peptide during constitutive processing of its precursor", *Science* 248:1122-1124 (1990);

35 Sisodia S. S. et al., "Evidence that  $\beta$ -amyloid protein in Alzheimer's disease is not derived by normal processing", *Science* 248:492-495 (1990)); it is estimated that ~20%

of the APP found on the membrane surface is released within 10 minutes (Koo, E. H. and S. L. Squazzo, "Evidence that production and release of amyloid  $\beta$ -protein involves the endocytic pathway", *J. Biol. Chem.* 109:991-998 (1996)). Of particular significance is the fact that this constitutively active  $\alpha$ -secretory pathway precludes the formation of  
5 intact A $\beta$  and, presumably, amyloid deposition.

The A $\beta$  peptide is secreted by cells (Haass, C. *et al.*, "Amyloid  $\beta$ -peptide is produced by cultured cells during normal metabolism", *Nature* 359:322-325 (1992); Seubert, P. *et al.*, "Isolation and quantitation of soluble Alzheimer  $\beta$ -peptide from biological fluids", *Nature* 359:325-357 (1992); Shoji, M. *et al.*, "Production of the  
10 Alzheimer amyloid  $\beta$  protein by normal and proteolytic processing", *Science* 258:126-129 (1992); Busciglio, J. *et al.*, "Generation of  $\beta$ -amyloid in the secretory pathway in neuronal and nonneuronal cells", *Proc. Natl. Acad. Sci. USA* 90:2092-2096 (1993)). It is believed that secreted A $\beta$  contributes to the deposition of insoluble amyloid in neuritic plaques (Selkoe, D. J., "Normal and abnormal biology of the  $\beta$ -amyloid precursor  
15 protein", *Annu. Rev. Neurosci.* 17:489-517 (1994)). Alternatively, A $\beta$  may accumulate intracellularly and thereby initiate the disease process (Wild-Bode *et al.*, "Intracellular generation and accumulation of amyloid  $\beta$ -peptide terminating at amino acid 42." *J. Biol. Chem.* 272:16085-16088 (1997)). As a result, considerable effort is underway to unravel the molecular pathways mediating A $\beta$  secretion. It appears that A $\beta$  can be  
20 generated both via a classical secretory pathway (Dyrks, T. *et al.*, "Amyloid precursor protein secretion and  $\beta$ A4 amyloid generation are not mutually exclusive", *FEBS Lett.* 349:210-214 (1994); Busciglio, J. *et al.*, "Generation of  $\beta$ -amyloid in the secretory pathway in neuronal and nonneuronal cells", *Proc. Natl. Acad. Sci. USA* 90:2092-2096 (1993); Citron, M. *et al.*, "Generation of amyloid  $\beta$  protein from its precursor is  
25 sequence specific", *Neuron* 14:662-670 (1995); Perez, R. G. *et al.*, "Enhanced release of amyloid  $\beta$ -protein from codon 670/671 "Swedish" mutant  $\beta$ -amyloid precursor protein occurs in both secretory and endocytic pathways", *J. Biol. Chem.* 271:9100-9107 (1996)), and after endocytosis of APP (Koo, E. H. and S. L. Squazzo, "Evidence that production and release of amyloid  $\beta$ -protein involves the endocytic pathway", *J. Biol.*  
30 *Chem.* 109:991-998 (1996); Higaki, J. *et al.*, "Inhibition of  $\beta$ -amyloid formation identifies proteolytic precursors and subcellular site of catabolism", *Neuron* 14:651-659 (1995); Perez, R. G. *et al.*, "Enhanced release of amyloid  $\beta$ -protein from codon 670/671 "Swedish" mutant  $\beta$ -amyloid precursor protein occurs in both secretory and endocytic pathways", *J. Biol. Chem.* 271:9100-9107 (1996)); however, many unknown details  
35 remain. Among these details is the mechanism by which A $\beta$  exits the cell.

Although secretion of both APP<sub>s</sub> and A $\beta$  is constitutive, the rate at which these two molecules are released from cells can be modified by activation of first and second

messenger systems. APP<sub>s</sub> secretion is increased (Buxbaum, J. D. *et al.*, "Processing of Alzheimer  $\beta$ /A4 amyloid precursor protein: Modulation by agents that regulate protein phosphorylation", *Proc. Natl. Acad. Sci. USA* 87:6003-6006 (1990); Caporaso, G. L. *et al.*, "Protein phosphorylation regulates secretion of Alzheimer  $\beta$ /A4 amyloid precursor protein", *Proc. Natl. Acad. Sci. USA* 89:3055-3059 (1992); Slack, B. E. *et al.*, "Regulation by phorbol esters of amyloid precursor protein release from Swiss 3T3 fibroblasts overexpressing protein kinase C $\alpha$ ", *J. Biol. Chem.* 268:21097-21101 (1993); Mills, J. and P. B. Reiner, "Phorbol esters but not the cholinergic agonists oxotremorine-M and carbachol increase release of the amyloid precursor protein in cultured rat cortical neurons", *J. Neurochem.* 67:1511-1518 (1996)) and A $\beta$  secretion is decreased (Buxbaum, J. D. *et al.*, "Protein phosphorylation inhibits production of Alzheimer amyloid  $\beta$ /A4 peptide", *Proc. Natl. Acad. Sci. USA* 90:9195-9198 (1993); Gabuzda, D. *et al.*, "Inhibition of  $\beta$ -amyloid production by activation of protein kinase C", *J. Neurochem.* 61:2326-2329 (1993); Hung, A. Y. *et al.*, "Activation of protein kinase C inhibits cellular production of the amyloid  $\beta$ -protein", *J. Biol. Chem.* 268:22959-22962 (1993); Jacobsen, J. S. *et al.*, "The release of Alzheimer's disease  $\beta$  amyloid peptide is reduced by phorbol treatment", *J. Biol. Chem.* 269:8376-8382 (1994)) by phorbol esters, presumably via activation of protein kinase C. Similar effects are seen following activation of membrane receptors thought to couple to protein kinase C (Buxbaum, J. D. *et al.*, "Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer  $\beta$ /A4 amyloid protein precursor", *Proc. Natl. Acad. Sci. USA* 89:10075-10078 (1992); Nitsch, R. M. *et al.*, "5-HT<sub>2a</sub> and 5-HT<sub>2c</sub> receptors stimulate amyloid precursor protein ectodomain secretion", *J. Biol. Chem.* 271:4188-4194 (1996); Nitsch, R. M. *et al.*, "Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors", *Science* 258:304-307 (1992); Lee, V. M. *et al.*, "Amyloid precursor protein processing is stimulated by metabotropic glutamate receptors", *Proc. Natl. Acad. Sci. USA* 92:8083-8087 (1995); Wolf, B. A. *et al.*, "Muscarinic regulation of Alzheimer's disease amyloid precursor protein secretion and amyloid  $\beta$ -protein production in human neuronal NT2N cells", *J. Biol. Chem.* 270:4916-4922 (1995), MAP kinase (Mills *et al.*, "Regulation of amyloid precursor protein catabolism involves the mitogen-activated protein kinase signal transduction pathway," *J Neurosci* 17:9415-9422 (1997); Desdouits-Magnen *et al.*, "Regulation of secretion of Alzheimer amyloid precursor protein by the mitogen-activated protein kinase cascade," *J Neurochem* 70:524-530 (1998)), as does activation of nerve growth factor receptors (Schubert, D. *et al.*, "The regulation of amyloid  $\beta$  protein precursor secretion and its modulatory role in cell adhesion", *Neuron* 3:689-694 (1989); Fukuyama, R. *et al.*, "Nerve growth factor-induced neuronal differentiation is



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- accompanied by differential induction and localization of the amyloid precursor protein (APP) in PC12 cells and variant PC12S cells", *Mol. Brain Res.* 17:17-22 (1993); Haring, R. *et al.*, "NGF promotes amyloid precursor protein secretion via muscarinic receptor activation", *Biochem. Biophys. Res. Comm.* 213:15-23 (1995)) and increasing
- 5 intracellular calcium (Buxbaum, J. D. *et al.*, "Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner", *Proc. Natl. Acad. Sci. USA* 91:4489-4493 (1994); Querfurth, H. W. and D. J. Selkoe, "Calcium ionophore increases amyloid beta peptide production by cultured cells", *Biochem.* 33:4550-4561 (1994)).
- 10 In summary, Alzheimer's disease, stroke, e.g., cerebral ischemia, and head injury are characterized by cognitive and neurological deficits associated with extracellular and cerebrovascular amyloid deposits.

#### Summary of the Invention

- 15 This invention provides methods, compositions, and screening methods which are useful in the treatment of amyloidosis. The methods of the invention involve administering to a subject a pharmaceutical composition including one or more agents which modulate (e.g., inhibit, prevent, or enhance) production and/or release of A $\beta$  and ultimately, amyloid deposition. Accordingly, the methods and compositions of the
- 20 invention are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs, e.g., Alzheimer's Disease, stroke and/or head trauma. The methods of the invention can be used therapeutically to treat amyloidosis or can be used prophylactically in a subject susceptible to amyloidosis. The methods of the invention are based, at least in part, on modulating cleavage of amyloid precursor protein (APP),
- 25 the proteolytic processing of APP and/or exportation of amyloid- $\beta$  protein (A $\beta$ ) by a blocker of a member of the ATP binding cassette (ABC) superfamily of transporters expressed in the brain or the cerebral microvasculature, e.g., *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.
- 30 Therefore, a therapeutic agent, such as a blocker, used in the method of the invention can modulate amyloid deposition.

- The present invention provides methods for modulating amyloid deposition in a subject, by administering to the subject an effective amount of an ATP binding cassette (ABC) transporter or flippase blocker. In one preferred embodiment, the modulation
- 35 includes preventing or inhibiting the amyloid deposition. The methods provide that one or more ABC transporter blockers or flippase blockers act to antagonize transport of A $\beta$

through one or more ABC transporters or flippase blockers expressed in the brain or the cerebral microvasculature.

The present invention also provides methods for treating a disease state associated with amyloid deposition by administering to a subject an effective amount of  
5 at least one ABC transporter or flippase blocker, or a pharmaceutically acceptable salt thereof, such that a disease state associated with amyloidosis is treated. In one preferred embodiment, the amyloid deposition is associated with Alzheimer's Disease.

The present invention also provides methods for treating Alzheimer's disease by administering to a subject having Alzheimer's disease an effective amount of at least one  
10 ABC transporter or flippase blocker, or a pharmaceutically acceptable salt thereof, such that treatment occurs.

The present invention pertains to methods for treating head trauma by administering to a subject having head trauma an effective amount of at least one ABC transporter or flippase blocker, or a pharmaceutically acceptable salt thereof, such that  
15 treatment occurs.

The present invention also pertains to methods for treating stroke by administering to a subject affected by a stroke an effective amount of at least one ABC transporter or flippase blocker, or a pharmaceutically acceptable salt thereof, such that treatment occurs.

20 The present invention further pertains to packaged pharmaceutical compositions for treating amyloidosis. A packaged pharmaceutical composition includes a container which holds an effective amount of a pharmaceutical composition for modulating amyloid deposition in a subject. The pharmaceutical composition includes at least one ABC transporter or flippase blocker and instructions for using the pharmaceutical  
25 composition. In one preferred embodiment, the packaged pharmaceutical composition is for treatment associated with Alzheimer's Disease.

The present invention also pertains to methods for identifying agents which modulate amyloid deposition in an organism by administering to an organism an effective amount of at least one ATP binding cassette (ABC) transporter or flippase  
30 blocker, such that modulation of amyloid deposition occurs. The methods provide that one or more ABC transporter blockers or flippase blockers which act to antagonize transport of A $\beta$  through one or more ABC transporters or flippase blockers expressed in the brain or the cerebral microvasculature can be identified.

The present invention further pertains to methods for identifying agents which  
35 modulate transport or flipping of amyloid across a membrane, e.g., a cellular or synthetic membrane. The methods include introducing an agent into a model system containing the membrane, e.g., cellular or planar lipid membrane, ABC transporter and amyloid.

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The ability of the agent to modulate the transport or flipping of amyloid across the membrane is measured. The methods provide the ability to identify of one or more ABC transporter blockers or flippase blockers which act to antagonize transport of A $\beta$  through one or more ABC transporters or ABC transporters having flippase activity which are expressed in the brain or the cerebral microvasculature.

#### **Brief Description of the Drawings**

FIG. 1 is a western blot of an SDS-PAGE gel demonstrating that RU-486 increases release of APP<sub>s</sub> from PC12 cells.

FIG. 2 is a graph comparing the effects of RU-486 and nerve growth factors upon APP<sub>s</sub> release from PC12 cells.

FIG. 3 is a western blot of an SDS-PAGE gel demonstrating that RU-486 increases release of APP<sub>s</sub> from PC12 cells in a dose-dependent manner.

FIG. 4 is a graph showing the dose-dependent effects of RU-486 administration upon APP<sub>s</sub> release from PC12 cells.

FIG. 5 is a western blot of an SDS-PAGE gel demonstrating that RU-486 increases release of APP<sub>s</sub> from PC12 cells after an exposure of only 15 minutes.

FIG. 6 is a western blot of an SDS-PAGE gel demonstrating that RU-486 increases release of APP<sub>s</sub> from E-82 cells which lack progesterone and glucocorticoid receptors.

FIG. 7 is a western blot of a SDS-PAGE gel demonstrating that RU-486, Progesterone and Desmethoxyverapamil increase APP<sub>s</sub> secretion in PC12 cells.

FIG. 8 is a graph of relative increase of APP<sub>s</sub> in Fig. 7.

FIG. 9 is a western blot of an SDS-PAGE gel demonstrating that RU-486 does not increase release of APP<sub>s</sub> from KB-3.1 cells which have minimal expression of p-glycoprotein.

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FIG. 10 is a western blot of an SDS-PAGE gel demonstrating that transfection of KB-3.1 cells with an expression vector for the human p-glycoprotein gene restores regulation of APP<sub>s</sub> release by RU-486.

FIG. 11 is a western blot of a SDS-PAGE gel demonstrating that RU-486  
5 decreases the release of A $\beta$  from SW cells.

FIG. 12 is a western blot of an SDS-PAGE gel demonstrating that RU-49953 decreases the release of A $\beta$  from SW cells.

10 FIG. 13 demonstrates transient transfection of P-glycoprotein increases basal secretion of  $\beta$ -amyloid.

FIG. 14 is a graphic depiction of densitometric analysis of transfected K269sw cells.  
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#### **Detailed Description of the Invention**

The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular  
embodiments of the invention are shown by illustration and not as limitations of the  
20 invention. The principal features of the invention can be employed in various embodiments without departing from the scope of the present invention. All parts and percentages are by weight unless otherwise stated.

The present invention pertains to methods for modulating amyloid deposition in a subject by administering to the subject an effective amount of at least one transport or  
25 flippase blocker for an ATP binding cassette (ABC) transporter which is expressed in the brain or cerebral microvasculature, such that modulation of amyloid deposition occurs. Amyloid deposition is a common problem associated with Alzheimer's disease, stroke and/or head trauma and is typically characterized by neuritic plaques. The present invention also pertains to methods for treating a disease state associated with  
30 amyloidosis by administering to a subject an effective amount of at least one ABC transporter blocker, or a pharmaceutically acceptable salt thereof, such that a disease state associated with amyloidosis is treated. The methods provide that one or more ABC transporter blockers or flippase blockers act to antagonize transport of A $\beta$  through one or more ABC transporters or flippase blockers expressed in the brain or the cerebral  
35 microvasculature.

The term ATP binding cassette (ABC) transporter is art recognized and includes the superfamily of transporters which use ATP as an energy source to transport various

molecules against concentration gradients across cell membranes. Currently, there are at least 33 members of the ABC superfamily of transporters in humans. Of these, at least 12 have been identified following complete or partial sequencing of the gene, with an additional 21 members of the superfamily identified as expressed-sequence tags (ESTs) (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Well-known examples of human ABC transporters include *MDR1* and the cystic fibrosis transmembrane regulator, although examples of ABC transporters are also well-known in other species such as the chloroquine transporter of *Plasmodium falciparum* and the yeast transporter *ste-6*. Of the 33 putative human ABC transporters, evidence suggests that at least 16 may be expressed in the brain or its microvasculature. Examples of human ABC transporters which are expressed in the brain and its microvasculature include *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5*, and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 and 422562.

The term "ABC transporter" is further intended to include a superfamily of genes found in many organisms including humans (Higgins CF, "ABC transporters: from microorganisms to man", *Annu Rev Cell Biol* 8:67-113 (1992)) characterized by a highly conserved region known as the ATP binding cassette (Hyde et al., "Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport", *Nature* 346:362-365 (1990); Mimura et al., "Structural model of the nucleotide binding conserved component of periplasmic permeases", *Proc Natl Acad Sci USA* 88:84-88 (1991)). These characteristic features provide a means of identifying unknown members of the ABC transporter superfamily using various database searching techniques, e.g. BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)). Using the N-terminal ATP-binding domain of *MDR1* as a conserved region of the superfamily of ABC transporters, this strategy has been utilized to identify a large family of ESTs corresponding to putative ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). The EST database can further be utilized as a first-pass means of examining expression of putative ABC transporters insofar as the expression library from which the EST derives can be used to provide partial information regarding the expression of the full-length gene. For example, an EST which is derived from an infant brain library is likely to be expressed in the brain.

The term "p-glycoprotein" is art recognized and is intended to include a subset of the family of ABC transporters. P-glycoproteins are large, glycosylated membrane

proteins that can function as ATP-dependent efflux pumps, and are encoded by two genes in humans known as *MDR1* and *MDR3* and three genes in the mouse known as *mdr1*, *mdr2*, and *mdr3* (Endicott, JA and Ling, V., "The biochemistry of p-glycoprotein-mediated multidrug resistance", *Ann Rev Biochem* 58:137-171 (1989)).

- 5       The term "*MDR1*" is art recognized and is intended to include the human *MDR1* encoded gene product (GenBank Accession No. M14758; Chen et al., "Internal duplication and homology with bacterial transport proteins in the *mdr1* (p-glycoprotein) gene from multidrug resistant human cells," *Cell* 47:381-389 (1986)) as well as its various isoforms as well as analogs, homologues, and orthologs in other species. The
- 10   *MDR1* gene product is an integral membrane protein having a predicted molecular weight of approximately 170 kDa, and has been shown to act as an ATP-dependent efflux pump with wide substrate specificity (Endicott, JA and Ling, V., "The biochemistry of p-glycoprotein-mediated multidrug resistance", *Ann Rev Biochem* 58:137-171 (1989)). The human *MDR1* gene is expressed in the cerebral
- 15   microvasculature and astrocytic foot processes (Thiebaut et al., "Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein p170: evidence for localization in brain capillaries and crossreactivity of one antibody with a muscle protein." *J Histochem Cytochem* 37:159-164 (1989); Pardridge et al., "Brain microvascular and astrocyte localization of p-glycoprotein," *J Neurochem*
- 20   68:1278-1285 (1997)).

- The term "*MDR3*" is art recognized and is intended to include the human *MDR3* encoded gene product (GenBank Accession No. M23234; Chen et al., "Internal duplication and homology with bacterial transport proteins in the *mdr1* (p-glycoprotein) gene from multidrug resistant human cells," *Cell* 47:381-389 (1986)) as well as its
- 25   various isoforms as well as analogs, homologues, and orthologs in other species. The *MDR3* gene product is an integral membrane protein and has been shown to promote translocation of phosphatidylcholine (Smith et al., "The human *MDR3* p-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice" *FEBS Lett* 354:263-266 (1994)). A BLAST (Altschul
- 30   et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human *MDR3* is encoded by ESTs corresponding to gb-AA677416, gb-AA456377, gb-AA459824, gb-W22853, and gb-R53330 (best 5 matches listed only). Because at least one of these ESTs derives from an infant brain library, *MDR3* is predicted to be expressed in brain.

- 35       The term "*ABC1*" is art recognized and is intended to include the human *ABC1* gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The mouse homologue of *ABC1* has been cloned (EMBL Accession No.

X75927, Luciani et al., "Cloning of two novel ABC transporters mapping on human chromosome 9," *Genomics* 21:150-159 (1994)) and may be involved in regulation of anion flux (Becq et al., "ABC1, an ATP binding cassette transporter required for phagocytosis of apoptotic cells, generates a regulated anion flux after expression in *Xenopus laevis* oocytes," *J Biol Chem* 272:2695-2699 (1997)), and secretion of interleukin 1 $\beta$  (Hamon et al., "Interleukin-1 $\beta$  secretion is impaired by inhibitors of the ATP binding cassette transporter, ABC1," *Blood* 90:2911-2915 (1997)). The human gene encoding ABC1 has been partially cloned, is found on chromosome 9, and is expressed in brain (Luciani et al., "Cloning of two novel ABC transporters mapping on human chromosome 9," *Genomics* 21:150-159 (1994)). A BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human ABC1 is encoded by ESTs corresponding to gb-U18236, gb-N46182, gb-H45142, gb-U66691, and gb-H21585 (best 5 matches listed only).

The term "ABC2" is art recognized and is intended to include the human ABC2 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The mouse homologue of ABC2 has been cloned (EMBL Accession No.X75927, Luciani et al., "Cloning of two novel ABC transporters mapping on human chromosome 9," *Genomics* 21:150-159 (1994)). The human gene encoding ABC2 has been partially cloned, is found on chromosome 9, and is expressed in brain (Luciani et al., "Cloning of two novel ABC transporters mapping on human chromosome 9," *Genomics* 21:150-159 (1994)). A BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human ABC2 is encoded by ESTs corresponding to gb-H39045, gb-U18235, gb-T33919, gb-W69928, and gb-M78056 (best 5 matches listed only).

The term "ABC3" is art recognized and is intended to include the human ABC3 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The cDNA for human ABC3 (GenBank Accession No. U78735, Connors et al., "The cloning of a human ABC gene (ABC3) mapping to chromosome 16p13.3," *Genomics* 39:231-234 (1997)) is 5112 nucleotides in length, encoding 1704 amino acids with a predicted molecular weight of 191 kDa, is found on chromosome 16p13.3, and is expressed in brain.

The term "ABC7" is art recognized and is intended to include the human ABC7 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The mouse homologue of ABC7 (*abc7*) has been partially cloned and is expressed in the brain (GenBank Accession No. U43892, Savary et al. "Isolation and chromosomal mapping of a novel ATP-binding cassette transporter conserved in mouse and human," *Genomics* 41: 275-278 (1997)). A BLAST (Altschul et al., "Basic local

alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human *ABC7* is encoded by ESTs corresponding to gb-U66679, gb-AA403130, gb-AA626765, gb-AA668992, gb-AA733151 (best 5 matches listed only).

The term "*ABC8*" is art recognized and is intended to include the human *ABC8* gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The gene encoding human *ABC8* has been cloned (GenBank Accession No. U34919, Croop et al., "Isolation and characterization of a mammalian homologue of the *Drosophila* white gene," *Gene* 185:77-85 (1997); GenBank Accession No. X91249, Chen et al., "Cloning of the cDNA for a human homologue of the *Drosophila* white gene and mapping to chromosome 21q22.3," *Am J Human Genetics*. 59:66-75 (1996), and predicts a protein of (at least) 638 amino acids in length, homologous to the *Drosophila* white gene, mapping to chromosome 21q22.3, and is expressed in brain. Because there is no stop codon prior to the putative initiation methionine, it is unclear as to whether this represents the complete or partial sequence of human *ABC8*. The mouse homologue of human *ABC8* (*abc8*) has been cloned and is also expressed in the brain (EMBL Accession No. Z48745, Savary et al. "Molecular cloning of a mammalian ABC transporter homologue to *Drosophila* white gene," *Mammalian Genome* 7: 673-676 (1996); gb-u34920, Croop et al., "Isolation and characterization of a mammalian homologue of the *Drosophila* white gene," *Gene* 185:77-85 (1997)). A BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human *ABC8* is encoded by ESTs corresponding to gb-AA297078, gb-AA297109, gb-AA305082, gb-AA297995, and gb-AA297906 (best 5 matches listed only).

The term "*MRP4*" is art recognized and is intended to include the human *MRP4* gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The human gene encoding *MRP4* has been partially cloned, is found on chromosome 13 (GenBank Accession No U83660, Kool et al., "Analysis of expression of cMOAT (*MRP2*), *MRP3*, *MRP4* and *MRP5*, homologues of the multidrug resistance-associate protein gene (*MRP1*), in human cancer cell lines," *Cancer Res* 57:3537-3547 (1997)). A BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human *MRP4* is encoded by ESTs gb-R35797, gb-U66686, gb-R35798, gb-N66654, and gb-AA015868 (best 5 matches listed only). Because at least one of these ESTs derives from an infant brain library, *MRP4* is predicted to be expressed in brain.

The term "*MRP5*" is art recognized and is intended to include the human *MRP5* gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. The human gene encoding *MRP5* has been partially cloned, is found on



chromosome 3, and is expressed in brain (GenBank Accession No. U83661, Kool et al., "Analysis of expression of cMOAT (*MRP2*), *MRP3*, *MRP4* and *MRP5*, homologues of the multidrug resistance-associate protein gene (*MRP1*), in human cancer cell lines," *Cancer Res* 57:3537-3547 (1997)). A BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of GenBank predicts that human *MRP5* is encoded by ESTs gb-U66687, gb-H17207, gb-AA829904, gb-H60893, gb-R34891 (best 5 matches listed only).

The term "human ABC transporter encoded by EST 45597" is art recognized and is intended to include the human EST 45597 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 45597 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of *MDR1* as a conserved region of superfamily of ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 45597 is found in an infant brain library, the human ABC transporter encoded by EST 45597 is predicted to be expressed in the brain.

The term "human ABC transporter encoded by EST 122234" is art recognized and is intended to include the human EST 122234 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 122234 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of *MDR1* as a conserved region of superfamily of ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 122234 is found in an infant brain library, the human ABC transporter encoded by EST 122234 is predicted to be expressed in the brain.

The term "human ABC transporter encoded by EST 123147" is art recognized and is intended to include the human EST 123147 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 123147 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of *MDR1* as a conserved region of superfamily of ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 123147 is found in an infant brain library,

the human ABC transporter encoded by EST 123147 is predicted to be expressed in the brain.

5 The term "human ABC transporter encoded by EST 131042" is art recognized and is intended to include the human EST 131042 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 131042 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of MDR1 as a conserved region of superfamily of ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and  
10 mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 131042 is found in an infant brain library, the human ABC transporter encoded by EST 131042 is predicted to be expressed in the brain.

15 The term "human ABC transporter encoded by EST 157481" is art recognized and is intended to include the human EST 45597 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 157481 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of MDR1 as a conserved region of superfamily of ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and  
20 mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 157481 is found in an infant brain library, the human ABC transporter encoded by EST 157481 is predicted to be expressed in the brain.

25 The term "human ABC transporter encoded by EST 182763" is art recognized and is intended to include the human EST 182763 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 182763 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of *MDR1* as a conserved region of superfamily of ABC transporters (Allikmets et al., "Characterization of the human ABC superfamily: isolation and  
30 mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 182763 is found in an infant brain library, the human ABC transporter encoded by EST 182763 is predicted to be expressed in the  
35 brain.

The term "human ABC transporter encoded by EST 352188" is art recognized and is intended to include the human EST 352188 gene product and its various isoforms

as well as analogs, homologues, and orthologs in other species. EST 352188 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of *MDR1* as a conserved region of superfamily of ABC transporters

- 5 (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 352188 is found in an infant brain library, the human ABC transporter encoded by EST 352188 is predicted to be expressed in the brain.

- 10 The term "human ABC transporter encoded by EST 422562" is art recognized and is intended to include the human EST 422562 gene product and its various isoforms as well as analogs, homologues, and orthologs in other species. EST 422562 was identified via BLAST (Altschul et al., "Basic local alignment search tool," *J Mol Biol* 215:403-410 (1990)) search of the public EST database using the N-terminal ATP-binding domain of *MDR1* as a conserved region of superfamily of ABC transporters
- 15 (Allikmets et al., "Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)). Because EST 422562 is reported to be a housekeeping gene (Allikmets et al., "Characterization of the human ABC superfamily: isolation and
- 20 mapping of 21 new genes using the Expressed Sequence Tags database", *Human Mol Genetics* 5:1649-1655 (1996)), the human ABC transporter encoded by EST 422562 is predicted to be expressed in the brain.

- The term "transporter blocker" is intended to include those compounds that can modulate ABC transporters expressed in the brain or the cerebral microvasculature, such
- 25 as those described *supra*, including *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 and 422562. The term "modulate" includes effect(s) on ABC transporters that prevent or inhibit amyloid production and/or release, which may ultimately affect amyloid deposition, e.g., in the context of the therapeutic
- 30 methods of the invention. In another embodiment, the term modulate includes effects on ABC transporters that enhances amyloid deposition, e.g., increase the production of amyloid in an animal model used to screen drugs for their ability to reduce amyloid deposition. For example, the blocker can affect an ABC transporter's ability to transport A $\beta$  extracellularly from a cell, modulate the cleavage of amyloid precursor protein
- 35 (APP), and modulate the proteolytic processing of APP. In one embodiment, the ABC transporter blocker is one or more lipophilic agents. In another embodiment, the blocker acts as a substrate for one or more ABC transporters.

The term "altered expression" includes effects upon the level of expression of either the mRNA or protein which encodes an ABC transporter.

Representative blockers useful in the present invention include, but are not limited to verapamil, desmethoxyverapamil, chloroquine, quinine, chinchonidine, primaquine, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, trifluoroperazine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1,9-dideoxyforskolin, cyclosporin, (US Patent 4,117,118 (1978)), PSC-833 (cyclosporin D, 6-[(2S, 4R, 6E)-4-methyl-2-(methylamino)-3-oxo-6-octenoic acid]-(9CI)), [US Patent 5,525,590] [ACS 121584-18-7], Keller et al., "SDZ PSC 833, a non-immunosuppressive cyclosporine: its potency in overcoming p-glycoprotein-mediated multidrug resistance of murine leukemia", *Int J Cancer* 50:593-597 (1992)), RU-486 (17 $\beta$ -hydroxy-11 $\beta$ -[4-dimethylaminophenyl]-17 $\alpha$ -prop-1-ynyl estro-4, 9-dien-3 one), RU-49953 (17 $\beta$ -hydroxy-11 $\beta$ , 17 $\alpha$ -[4-dimethylaminophenyl] - 17 $\alpha$  prop-1-ynyl estro-4, 9 dien-3 one), S9778 (6-{4-[2,2-di( )ethylamino]-1-piperidinyl}-N,N', di-2-propenyl-1,3,5-triazine-2,4-diamine, bismethane sulfonate, [US patent 5,225,411; EP 466586] [ACS # 140945-01-3]; Dhainaut et al., "New triazine derivatives as potent modulators of multidrug resistance," *J Medicinal Chemistry* 35:2481-2496 (1992)), MS-209 (5-[3-[4-(2,2-diphenylacetyl)piperazin-1-yl]-2-hydroxypropoxy]quinoline sesquifumarate, [US patent 5,405,843 (continuation of 5,112,817)], [ACS # 158681-49-3], Sato et al., "Reversal of multidrug resistance by a novel quinoline derivative, MS-209, *Cancer Chemother Pharmacol* 35:271-277 (1995)), MS-073 (Fukazawa et al., European Patent Application 0363212 (1989)), FK-506 (Tanaka et al., M. Physicochemical properties of FK-506, a novel immunosuppressant isolated from *Streptomyces tsukubaensis*" *Transplantation Proceedings*. 19(5 Suppl 6):11-6, (1987); Naito et al., "Reversal of multidrug resistance by an immunosuppressive agent FK-506," *Cancer Chemother & Pharmacol.* 29:195-200 (1992); Pourtier-Manzanedo et al., "FK-506 (fujimycin) reverses the multidrug resistance of tumor cells in vitro," *Anti-Cancer Drugs* 2:279-83 (1991); Epand & Epand, "The new potent immunosuppressant FK-506 reverses multidrug resistance in Chinese hamster ovary cells," *Anti-Cancer Drug Design* 6:189-93 (1991)), VX-710 (2-peperidinecarboxylic acid, 1-[oxo(3,4,5-trimethoxyphenyl)acetyl]-3-(3-pyridinyl)-1-[3-(3-pyridinyl)propyl]butyl ester [ACS 159997-94-1] [US patent number 5,620,971] Germann et al., "Chemosensitization and drug accumulation effects of VX-710, verapamil, cyclosporin A, MS-209 and GF120918 in multidrug resistance-associated protein MRP" *Anti-Cancer Drugs* 8, 141-155 (1997) ; Germann et al., "Cellular and biochemical characterization of VX-710 as a chemosensitizer: reversal of P-glycoprotein-mediated multidrug resistance in vitro" *Anti-Cancer Drugs* 8, 125-140

(1997)), VX-853 ([US patent number 5,543,423] [ACS # 190454-58-1), AHC-52 (methyl 2-(N-benzyl-N-methylamino)ethyl-2, 6-dimethyl-4-(2-isopropylpyrazolo[1,5-a]pyridine-3-yl)-1,4-dihydropyridine-3,5-dicarboxylate; [Japanese Patent 63-135381; European Patent 0270926] [ACS 119666-09-0] Shinoda et al., "In vivo circumvention of vincristine resistance in mice with P388 leukemia using a novel compound, AHC-52," *Cancer Res* 49:1722-6 (1989)), GF-120918 (9,10-dihydro-5-methoxy-9-oxo-N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxyisoquinol-2-yl) ethyl]phenyl]-4 acridinecarboxamide,[US patent 5,604,237] [ACS # 143664-11-3] Hyafil et al., "In vitro and in vivo reversal of multidrug resistance by GF120918, an acridonecarboxamide derivative," *Cancer Res* 53:4595-4602 (1993)), and XR-9051 (3-[(3Z, 6Z)-6-Benzylidene-1-methyl-2,5-dioxopiperazin-3-ylidenemethyl]-N-[4-[2-(6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]phenyl]benzamide hydrochloride, [ACS#57-22-7]).

In a preferred embodiment, blockers useful in the present invention include RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propranolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine. In another embodiment, blockers useful in the present invention include RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propranolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine. In one embodiment, the ABC transporter blocker is not RU-486. Preferred blockers include PSC 833, MS-209, VX-710, VX-853, GF 120918, XR-9051, S 9788 and RU-49953.

RU-486 is generally associated with steroid hormone receptors such as glucocorticoid and progesterone receptors. For example, RU-486 acts as an antagonist toward these receptors and most commonly is used to block their ability to alter gene transcription (Moguilewsky M. and Philbert D., RU38486: "Potent anti-glucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by impaired activation." *J. Steroid Biochem.* 20:271-276(1984); Meyer *et al.* "Agonistic and antagonistic activities of RU-486 on the functions of the human progesterone receptor." *EMBO J.* 9:3923-3932 (1990)). "Steroid hormone receptor agonists and antagonists such as RU-486 are also able to act in a "nongenomic" fashion, altering

tyrosine phosphorylation, neurotransmitter release, the concentration of intracellular calcium, inositol triphosphate production, levels of intracellular cAMP, and membrane potential." (Wehling, M. "Nongenomic actions of steroid hormones", *Trends Endocrinol. Metab.* 5:347-353, (1994)). RU-486 also causes dissociation of the hetero-  
5 oligomeric complex of proteins to which unliganded steroid hormone receptors are bound resulting in release and activation of steroid hormone receptor associated proteins (Lebeau, M. C. and Baulieu, E. E., Steroid antagonists and receptor-associated proteins, *Human Reproduction* 9(Suppl)2:11-21 (1994)). Steroid hormone receptors and  
10 structurally related antagonists also are known to act upon numerous membrane proteins, in particular neurotransmitter receptors (Brann D. W. *et al.* Emerging diversities in the mechanism of action of steroid hormones, *J. Steroid Biochem. Mol. Biol.* 52:113-133, (1995)).

RU49953 (17b-hydroxy-11b, 17a-[4-dimethylaminophenyl]- 17a prop-1-ynyl  
15 estra-4, 9-dien-3 one) is a derivative of the antiglucocorticoid/antiprogesterin RU486 (17b-hydroxy-11b-[4-dimethylaminophenyl]-17a prop-1-ynyl estra-4, 9-dien-3 one) which does not bind to either glucocorticoid or progesterone receptors to any appreciable degree (Marsaud V, Mercier-Bodard C, LeBihan S, Renoir JM, "Dexamethasone and triamcinoloneacetone uptake by mouse fibroblasts is differently modulated by the immunosuppressants cyclosporin A, FK506, rapamycin and their analogues, as well as  
20 by other p-glycoprotein ligands", *J Steroid Biochem Mol Biol* (in press)), however they do bind to and block MDR1 mediated efflux (Jault, Renoir, & DiPietro, personal communication cited in Marsaud et al., in press).

The term "disease state" is intended to include those diseases, disorders or conditions which are associated with an increased amount of amyloid deposition,  
25 relative to a subject not afflicted with the disease, disorder or condition, in that the deposition of amyloid is directly or indirectly a causative agent of the disease, disorder or condition. The amyloid deposition does not have to be the sole causative agent of the disease, disorder or condition but be merely responsible for causing some of the symptoms typically associated with the disease, disorder, or condition being treated.  
30 Amyloid deposition can be the causative agent alone or at least one other agent can be involved in the state being treated. Examples include Alzheimer's Disease, head trauma, stroke, e.g., cerebral ischemia, Down's syndrome, hereditary cerebral hemorrhage amyloidosis, familial Mediterranean Fever, familial amyloid nephropathy with urticaria and deafness [Muckle-Wells syndrome], myeloma or macroglobulinemia, chronic  
35 hemodialysis, familial amyloid polyneuropathy, familial amyloid cardiomyopathy, adult onset diabetes, insulinoma, gelsolin, cystatin C (hereditary cerebral hemorrhage with amyloidosis), familial amyloidotic polyneuropathy, Scrapie, Creutzfeldt-Jacob disease,

kuru, Gerstmann-Straussler-Scheinker syndrome, bovine spongiform encephalopathy. Preferred examples include those symptoms associated with Alzheimer's Disease, stroke and head trauma

The present invention provides methods for treating head trauma by  
5 administering to a subject having head trauma an effective amount of at least one ABC transporter or flippase blocker, or a pharmaceutically acceptable salt thereof, such that treatment occurs.

The present also provides methods for treating stroke by administering to a subject affected by a stroke an effective amount of at least one ABC transporter or  
10 flippase blocker, or a pharmaceutically acceptable salt thereof, such that treatment occurs.

The term "lipophilic agent," as the term is used herein, refers to a compound, such as a therapeutic agent, which, as a separate entity, is more soluble in nonpolar solvents than water. For example, verapamil is considered lipophilic because it has  
15 greater solubility in hexane than in water.

The lipophilic nature of a lipophilic agent is related to its structure. For example, the agent can include lipophilic substituents such as saturated or unsaturated, substituted or unsubstituted alkyl, aryl or heteroaryl groups. Such groups include substituted and unsubstituted, normal, branched or cyclic alkyl groups having 3 or more carbon atoms,  
20 substituted or unsubstituted arylalkyl or heteroarylalkyl groups and substituted or unsubstituted aryl or heteroaryl groups.

Further, the lipophilic nature of the therapeutic agent can also be attributed to the "backbone" of the compound. The backbone of the compound is that portion of the structure to which substituents are attached and can include lipophilic groups such as  
25 steroidal groups, saturated or unsaturated, substituted or unsubstituted alkyl, aryl or heteroaryl groups, or other fused cyclic ring systems.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In one  
30 embodiment, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C<sub>1</sub>-C<sub>30</sub> for straight chain, C<sub>3</sub>-C<sub>30</sub> for branched chain), and more preferably 20 or fewer. Likewise, cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term alkyl as used throughout the specification and claims is  
35 intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example,

halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxy alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino),  
5 acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if  
10 appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "aralkyl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)).

The term "aryl" as used herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole,  
15 furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more  
20 ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino),  
25 acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g.,  
30 tetralin).

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Preferred alkyl groups are lower alkyls having one to three carbon atoms.

35 The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 4- to 7-membered rings, which ring structures include one to four heteroatoms. Heterocyclyl groups include pyrrolidine, oxolane, thiolane, oxazole,



piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, lactones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, 5 aryloxy carbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, 10 sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. A heteroalkyl moiety is an alkyl substituted with a heteroaromatic group.

The terms "polycyclyl" or "polycyclic group" refer to two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two 15 or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, 20 aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylaryl amino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, 25 heterocyclyl, alkyl, aralkyl, or an aromatic or heteroaromatic moiety.

The term "substrate" as used herein means the specific compound acted upon by an enzyme, transporter, or other cellular proteins.

The language "modulation of amyloid deposition" as used herein, means that amyloid deposition is prevented or decreased, e.g. A $\beta$  deposition. This modulation can 30 be by one or more chemically induced physiological mechanisms. For example, the blocking agents and/or the lipophilic agents of the present invention can modulate amyloidosis in a subject such as by acting as an ATP binding cassette (ABC) transporter blocker, more preferably as a blocker of an ABC transporter expressed in the brain or cerebral microvasculature as described *supra*. For example the blockers and/or the 35 lipophilic agents of the present invention can modulate the cleavage of amyloid precursor protein (APP). In one embodiment the blocker and/or lipophilic agent can modulate proteolytic processing of APP, thereby decreasing production of amyloid- $\beta$

protein (A $\beta$ ). In yet another embodiment, the blockers and/or the lipophilic agents of the present invention modulate proteolytic processing of APP, thereby increasing production of soluble amyloid precursor protein (APP<sub>s</sub>). Additionally, the blockers and/or lipophilic agents of the present invention can modulate an ABC transporter's ability to export A $\beta$  from a cell. Most preferably, the blockers and/or lipophilic agents of the present invention inhibit or prevent export of A $\beta$  from a cell.

Alternatively, the term "modulate" is intended to mean that amyloid deposition is increased, e.g. A $\beta$  deposition. This modulation can be induced by one or more chemically induced physiological mechanisms. For example, effective amounts of a chemical agent can be screened for increased amyloid deposition in a subject. The increase can be evaluated by comparing a subject treated with an agent of the present invention to a similar subject who was not treated. Furthermore, in some embodiments of the invention, the subject can have pre-existing amyloid deposits; therefore, the chemical agent serves to enhance the existing deposits. Preferably the increase is at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to an untreated subject.

The term "subject" is intended to include mammals having amyloid deposition, including one or more amyloid related symptoms, or which are susceptible to amyloid deposition. Examples of such subjects include humans, dogs, cats, pigs, cows, horses, rats and mice.

The term "administering" is intended to include routes of administration which allow the ABC transporter blocker and/or the lipophilic agent to perform its intended function, e.g., preventing or inhibiting amyloidosis. A variety of routes of administration are possible including, but not necessarily limited to parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), oral (e.g., dietary), topical, nasal, rectal, or via slow releasing microcarriers depending on the disease or condition to be treated. Oral, parenteral and intravenous administration are preferred modes of administration. Formulation of the compound to be administered will vary according to the route of administration selected (e.g., solution, emulsion, gels, aerosols, capsule). An appropriate composition comprising the compound to be administered can be prepared in a physiologically acceptable vehicle or carrier and optional adjuvants and preservatives. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media, sterile water, creams, ointments, lotions, oils, pastes and solid carriers. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include

various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. (1980)).

The language "effective amount" is that amount of the blocker which allows it to perform its intended function. For example, an effective amount is that amount  
5 sufficient to inhibit, partially or totally, amyloid release, production and/or deposition or to reverse amyloid deposition or prevent or reduce its further deposition. The "effective amount" also includes the amount sufficient to treat amyloidosis or Alzheimer's disease. The effective amount will depend upon a number of factors, including biological activity of the blocker and/or lipophilic agent, age, body weight, sex, general health, severity of  
10 the disease to be treated, as well as appropriate pharmacokinetic properties. For example, dosages of the active substance can be from about 10 mg/kg/day to about 1000 mg/kg/day. A therapeutically effective amount of the active substance can be administered by an appropriate route in a single dose or multiple doses. Further, the dosages of the active substance can be proportionally increased or decreased as indicated  
15 by the exigencies of the therapeutic or prophylactic situation.

The term "amyloidosis" is art recognized and is intended to include amyloid deposition related symptoms, such as progressive and undesirable memory impairment, loss of language and visuospatial skills, and behavior deficits. These changes in cognitive function are likely the result of degeneration of neurons in the cerebral cortex,  
20 hippocampus, basal forebrain, and other regions of the brain. The presence of large numbers of neurofibrillary tangles in degenerated neurons, neuritic plaques in the extracellular space and in the walls of the cerebral microvasculature are thought to be a result of amyloid deposition. For example, neuritic plaques consist of deposits of proteinaceous material surrounding an amyloid core.

The language "pharmaceutically acceptable salt" is intended to include pharmaceutically acceptable salts capable of being solvated under physiological conditions. Examples of such salts include sodium, disodium, potassium, dipotassium, and hemisulfate. The term is further intended to include lower hydrocarbon groups capable of being solvated under physiological conditions, e.g. alkyl esters, methyl, ethyl  
30 and propyl esters.

The present invention further pertains to packaged pharmaceutical compositions for treating amyloidosis. The package includes a container for holding an effective amount of a pharmaceutical composition and instructions for using the pharmaceutical composition for treatment of amyloidosis. The pharmaceutical composition includes at  
35 least one ABC transporter blocker for modulating amyloid deposition in a subject.

The term "pharmaceutical composition" includes blockers and/or lipophilic agents of the present invention and includes ingredients, such as other therapeutically

active substances, inert ingredients, and carrier compounds. The components of the composition must be compatible, meaning that the components must be capable of being commingled with the active substance, e. g. the blocker and/or the lipophilic agent and with each other in a manner such that there is no interaction which would substantially  
5 reduce during use the composition's efficacy for modulating amyloid deposition.

The pharmaceutical compositions can be prepared by known procedures using well known and readily available ingredients. In making the pharmaceutical compositions of the present invention, the active substance will usually be admixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of  
10 a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, (as a solid or in a liquid medium), ointments containing up to 10% by weight  
15 of the active compound, soft and hard gelatin capsules, packaged powders, and the like. Examples of suitable carriers, excipients, and diluents are lactose, dextrose, sucrose, sorbitol, mannitol, starches, gumacacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methylhydroxybenzoates, propylhydroxybenzoates,  
20 propylhydroxybenzoates, talc, and other compounds as are well known to those skilled in the pharmaceutical arts.

Blockers and/or lipophilic agents of the invention can be administered alone or in conjunction with other pharmacologically active agents, e.g., together with immunosuppressive agents or together with antibiotics and/or antiviral agents.  
25 Compounds that can be coadministered include steroids (e.g. methyl prednisolone acetate), NSAIDs and other known immunosuppressants such as azathioprine, 15-deoxyspergualin, mizoribine, mycophenolate mofetil, brequinar sodium, leflunomide, and related molecules. Dosages of these drugs will also vary depending upon the condition and individual to be treated.

30 Blockers and/or lipophilic agents of the invention can be administered prior to the onset of amyloidosis, or after the onset of amyloidosis. The blockers and/or lipophilic agents also can be administered as a prodrug which is converted to another form *in vivo*.

The present invention also pertains to methods for identifying agents which  
35 modulate amyloid production in an organism by administering to an organism, e.g., a transgenic mouse model which overexpresses A $\beta$ , resulting in amyloid deposits, an effective amount of at least one ATP binding cassette (ABC) transporter blocker, such

that modulation of amyloid deposition occurs. The observation that amyloid production, release or deposition occurs in membrane vesicles, cells, or organisms is used to identify the agents. In another embodiment of the present invention, either the ABC transporter blocker or another agent induces a change in ABC transporter expression or stability which enhances amyloid production and can be used to produce a model for amyloidosis; in one embodiment, the model is an animal model, although other organisms, cell lines and even membranes may be useful in this regard. These models can be used to screen agents or drugs for their ability to reduce or prevent amyloid deposits.

10 The term "organism" is intended to include single cells, such as *E. coli*, multicellular organisms such as yeast and *C. elegans* cell lines and multicellular organisms including mammals, such as mice, rats, guinea pigs, and pigs that can develop amyloidosis. Examples of suitable multicellular organisms include transgenic animals, e.g., mammals, as well as those mammals identified as capable of developing amyloidosis, e.g., having amyloid deposition.

15 The term "method for identifying agents" includes assays and the like suitable for determining what agent or agents (blockers and/or lipophilic agents) elicit a response by the assay method. For example, combinatorial libraries can be screened to determine whether any members of the library have a desired activity, and, if so, to identify the active species. Methods of screening combinatorial libraries have been described (see, 20 e.g., Gordon *et al.*, *J Med. Chem.*, *op. cit.*). Soluble compound libraries can be screened for modulating transport of amyloid across cell membrane, followed by identification of the isolated compounds by conventional techniques (e.g., mass spectrometry, NMR, and the like). Preferably, the library compounds are conjugated to a label (e.g., 25 fluorophores, colorimetric enzymes, radioisotopes, luminescent compounds, and the like) that can be detected to indicate reduction in ABC transport of amyloid. Alternatively, immobilized compounds can be selectively released and allowed to interact with an ABC transporter. Exemplary assays useful for screening the libraries of the invention are known in the art (see, e.g., E. M. Gordon *et al.*, *J. Med. Chem.* 30 37:1385-1401 (1994)).

For example, cell lines can be developed which over-express each member of the family of ABC transporters, preferably, those ABC transporters expressed in the brain and/or the cerebral microvasculature, e.g., *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562, thereby providing 35 surfaces of such cells with thousands of copies of a particular ABC transporter (For similar techniques see: Shapiro, A. B. and Ling, V., Reconstitution of Drug Transport by

Purified P-Glycoprotein, *Journal of Biological Chemistry*, 270: 16167-16175, (1995)). From these cells, inside-out plasma vesicles can be constructed, thereby externalizing all of the molecular architecture found inside cells. Addition of ATP to these vesicles, causes the ABC transporter to move substrates across the cell membrane, except rather than moving substrates from inside (intracellular) to outside (extracellular), the substrate now becomes concentrated inside the vesicle. Reliable preparations of plasma vesicles that transport amyloid can be used to screen chemical libraries to isolate new compounds capable of reducing ABC transport of amyloid.

The present invention further pertains to *in vitro* and *in vivo* methods for identifying agents which modulate transport of amyloid across membranes, e.g., cellular or synthetic membranes. The methods include introducing an agent into a model system which contains a membrane, an ABC transporter expressed in the brain or cerebral microvasculature and amyloid. The ability of the agent to modulate the transport of amyloid across the membrane is measured. Suitable synthetic membranes are those composed of lipids as described in (Slatin, S. "Colicin E in planar lipid bilayers" *Internat. J. Biochem.* 20:737-744 (1988); Krasilnikov *et al.*, "A novel approach to study the geometry of the water lumen of ion channels: colicin 1a in planar lipid bilayers" *J. Memb. Biol.* 161:83-92 (1998)).

The present invention also pertains to *in vitro* methods for inserting ABC transporters, preferably those ABC transporters expressed in the brain and/or cerebral microvasculature, e.g., *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562, into planar lipid bilayers as a means of assaying regulation of amyloid transport. For example, purified or recombinant proteins can be inserted into planar lipid bilayers (Slatin, S. "Colicin E in planar lipid bilayers" *Internat. J. Biochem.* 20:737-744 (1988); Krasilnikov *et al.*, "A novel approach to study the geometry of the water lumen of ion channels: colicin 1a in planar lipid bilayers" *J. Memb. Biol.* 161:83-92 (1998)) such that transport of amyloid across the planar lipid bilayer by the ABC transporter can be measured. The methods include introducing an agent into a model system which contains a planar lipid bilayer, an ABC transporter and amyloid. The ability of the agent to modulate the transport of amyloid across the planar lipid bilayer can be measured.

The term "model system" includes cells, cell lines, mammals, birds, insects, single- and multicellular organisms and *in vitro* systems.

The following discussion, which is not to be construed as limiting on the invention in any way, but is presented for purposes of illustration, describes a plausible mechanism for the modulation of cellular trafficking and proteolytic processing of APP.

Immature APP is believed to be transported through intracellular secretory machinery to the membrane where it is found as a transmembrane protein known as mature APP. During this maturation process, APP can be cleaved by  $\beta$ - and  $\gamma$ -secretases; the cleavage occurs at the N- and C-termini of A $\beta$  (respectively), resulting in production of A $\beta$  peptide. Once at the membrane, mature APP undergoes one of several proteolytic processing routes. The  $\alpha$ -secretory processing of APP involves cleavage of the molecule in the extracellular domain, near the membrane and within the A $\beta$  sequence. This cleavage of APP has two potential consequences: (1) it precludes formation of A $\beta$ ; and (2) solubilizes the extracellular domain of APP which is then released into the extracellular space. An alternative route involves internalization to an endosomal compartment with potential for  $\beta$ - and  $\gamma$ -secretase cleavage, once again resulting in production of A $\beta$ .

It is also believed that certain ABC transporters export A $\beta$  from cells. *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562 are members of the ATP-binding cassette (ABC) superfamily of transporters; using ATP as an energy source, certain ABC transporters such as *MDR1* are known to pump various molecules against their concentration gradients (Endicott, J. A. and V. Ling, "The biochemistry of P-glycoprotein-mediated multidrug resistance", *Annu. Rev. Biochem.* 58:137-171 (1989); Gottesman, M. M. and I. Pastan, "Biochemistry of multidrug resistance mediated by the multidrug transporter", *Annu. Rev. Biochem.* 62:385-342 (1993), the teachings of which are hereby incorporated by reference). In humans, two isoforms of p-glycoprotein exist; only one of them, termed *MDR1*, confers the multidrug resistance phenotype. In this document, the term p-glycoprotein is used to indicate the *MDR1* gene product. Other isoforms of p-glycoprotein as well as other members of the superfamily of ABC transporters can also serve to transport A $\beta$  from cells, and therefore can also be targets for the drugs listed below as "active compounds". A wide assortment of drugs act as substrates for *MDR1*; while structurally unrelated, all share the property of being exceedingly hydrophobic (Zamora, J. M. *et al.*, "Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemia cells", *Mol. Pharmacol.* 33:454-462 (1988), the teachings of which are hereby incorporated by reference) which has led to the belief that *MDR1* act as either a "hydrophobic vacuum cleaner" for a wide variety of membrane-associated hydrophobic molecules or as a flippase (Higgins, C. F. and M. M. Gottesman, "Is the multidrug transporter a flippase?", *TIBS* 17:18-21 (1992), the teachings of which are hereby incorporated by reference). Among the molecules which can be exported are lipophilic peptides (Raymond, M. *et al.*, "Functional complementation of yeast *ste6* by a

mammalian multidrug resistance *mdr* gene", *Science* 256:232-234; Sharma, R. C. et al., "Peptide transport by the multidrug resistance pump", *J. Biol. Chem.* 267:5731-5734 (1992), the teachings of which are hereby incorporated by reference). Because A $\beta$  is a small, lipophilic peptide, it has appropriate properties to act as a substrate for *MDR1*.

5       The term "flippase" is art recognized and is intended to include the ability of an ABC transporter to act as a flipping agent, e.g., to move A $\beta$  from the inner leaflet of a lipid bilayer to the outer leaflet. That is, an ABC transporter acting as a flippase delivers A $\beta$  to the outer leaflet of the lipid bilayer, in this scenario, other molecules may be involved in moving A $\beta$  from the outer leaflet of the lipid bilayer into the extracellular  
10 space. However, the flippase action of the ABC transporter is critical to the multi-factorial process leading to A $\beta$  efflux.

      The term "flippase blocker" is intended to include those compounds that can modulate ABC transporters expressed in the brain or the cerebral microvasculature, such as those described *supra*, including *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*,  
15 *MRP4*, *MRP5* and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.

      The sequence similarities between *MDR1* and other members of the superfamily of ABC transporters (Dean and Allikmets, "Evolution of ATP-binding cassette transporter genes" *Curr Opin Genetics Dev* 5:779-785 (1995)) suggest that they too may  
20 modify A $\beta$  efflux from cells. This is a reasonable inference, given what is known about the function of members of the family which are already characterized. For example, the yeast ABC transporter known as *ste-6* is responsible for export of a peptide called a-factor: yeast which are deficient in *ste-6* lack a-factor export, but replacement of *ste-6* with either the ABC transporter *mdr3*, a mouse homologue of human *MDR1* (Raymond  
25 et al., "Functional complementation of yeast *ste6* by a mammalian multidrug resistance *mdr* gene" *Science* 256:232-234 (1992)) or the human ABC transporter *MRP1* (Ruetz et al., "Functional expression of the multidrug resistance-associated protein in the yeast *Saccharomyces cerevisiae*" *J Biol Chem* 271:4154-4160 (1996)) restores a-factor export. These data demonstrate (a) that multiple members of the family indeed act as  
30 transporters; and (2) that the substrates which they transport are not unique to any given family member. Further evidence for the notion that ABC transporters indeed function as transporters in cells include the observation that the human *MDR3* gene product promotes translocation of phosphatidylcholine (Smith et al., "The human *MDR3* p-glycoprotein promotes translocation of phosphatidylcholine through the plasma  
35 membrane of fibroblasts from transgenic mice" *FEBS Lett* 354:263-266 (1994)), that the ABC transporter known as *abc1* appears to be involved in secretion of interleukin 1 $\beta$  (Hamon et al., "Interleukin-1 $\beta$  secretion is impaired by inhibitors of the ATP binding



cassette transporter, *ABC1*," *Blood* 90:2911-2915 (1997)), and that the transport of certain peptides into the lumen of the endoplasmic reticulum is accomplished by the conjoint efforts of two members of the ABC transporter family known as *TAP1* and *TAP2* (Heemels MT and Ploegh H, "Generation, translocation, and presentation of MHC class I-restricted peptides." *Ann Rev Biochem.* 64:463-91 (1995)).

$A\beta$  is released from all cells, and thus it is reasonable to assume that all cells have a mechanism for  $A\beta$  release. In cells expressing *MDR1*, it is believed that this ABC transporter is involved in the process of  $A\beta$  efflux. However, other than the microvasculature, there is little or no *MDR1* expression in the brain, yet neurons produce and release prodigious amounts of  $A\beta$  (Busciglio et al., "Generation of  $\beta$ -amyloid in the secretory pathway in neuronal and nonneuronal cells," *Proc Natl Acad Sci USA* 90:2092-2096 (1993); Simons et al., "Amyloidogenic processing of the human amyloid precursor protein in primary cultures of rat hippocampal neurons," *J Neurosci* 16:899-908 (1996)). Thus, it seems reasonable to propose that other ABC transporters are involved in the process of  $A\beta$  efflux in brain. As amyloid deposition in Alzheimer's disease occurs both in the parenchyma of the brain and in the microvasculature (Selkoe, DJ "The molecular pathology of Alzheimer's disease," *Neuron* 6:487-498 (1991)), ABC transporters expressed in brain and the microvasculature represent key targets for regulating  $A\beta$  release via the present invention. Thus, brain-and microvasculature-expressing ABC transporters represent preferred targets for the development of Alzheimer's disease therapeutics.

It is also possible that the ABC transporters *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* and the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562 can function to allosterically modify the function of other membrane proteins. In some cells, modulation of p-glycoprotein by ABC transporter blockers has been shown to alter the magnitude of volume-activated chloride currents (reviewed in Higgins, C. F. Volume-activated chloride currents associated with the multidrug resistance P-glycoprotein, *J. Physiol.* 482:31S-36S (1995)). In this model, p-glycoprotein and other ABC transporters have multiple functions, one of which is to allosterically modify the function of the other membrane proteins. The present invention is consistent with a model in which allosteric modification of other membrane proteins by an ABC transporter is responsible for the change in APP catabolism, such that  $A\beta$  release is reduced, and/or in which the ABC transporter exports  $A\beta$ . From this, it follows that pharmacological agents which alter the ability of these molecules to export  $A\beta$  or the expression of such molecules can be therapeutically useful in Alzheimer's disease.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references, pending patent applications and published patent applications, cited throughout this application are hereby incorporated by reference. It should be understood that the animal cell line models used throughout the examples are accepted cell models and that the demonstration of efficacy in these cell models is predictive of efficacy in humans.

### Exemplification

#### 10        I. The Use of a P-glycoprotein Blocker For Increasing APP<sub>s</sub> Secretion

Murine nerve growth factor (NGF) was purchased from GIBCO and dexamethasone (DEX) was purchased from SIGMA. Mifepristone (RU-486) was purchased from Research Biochemicals International (RBI, Natick, MA) through the NIMH (National Institute for Mental Health) Chemical Synthesis Program. Cyclosporin A (CsA) was purchased from RBI. The Anti-Alzheimer Precursor Protein A4 (22C11) monoclonal antibody specific to the pre-A4 molecule (amyloid precursor protein) was purchased from Boehringer Mannheim.

20        The GSrasDN1 PC12 subline (Dr. Simon Halegous (State University of New York at Stony Brook)) expressing the dominant inhibitory mutant *ras* gene, p21<sup>N17</sup> under the control of a dexamethasone-inducible MMTV promoter (mouse mammary tumor virus), obtained as a gift from Dr. Simon Halegoua, (Kremer *et al.*, J. Cell Biol. 115(3):809-819 (1991)) and grown in Dulbecco's modified Eagles' medium (DMEM, GIBCO) containing 5% fetal bovine serum (FBS: v/v) and 10% heat inactivated horse serum (HS, GIBCO).

25        RU-486 and NGF were solubilized in dimethylsulfoxide (DMSO). DMSO was used as a control vehicle in all experiments.

For drug exposure experiments in GSrasDN1 PC12 cells, cells were grown to confluence in 75 cm<sup>2</sup> polypropylene culture flasks from Falcon. Twelve hours prior to drug treatment, the culture medium was aspirated using a pasteur pipette and replaced with 10 ml of DMEM supplemented with 15% charcoal-stripped calf serum (SIGMA) to remove complement, immunoglobulins and endogenous steroid hormones secreted from the cells. Twelve hours later, this supplemental medium was aspirated and replaced with 10 ml of DMEM containing the treatment drugs. Flasks were allotted into the following treatment groups: a) Control and b) RU-486 (3.0 μM). DMSO concentrations were normalized to 0.06% (v/v) in all flasks.

Twelve hours following drug exposure, the medium was again aspirated and the cells were washed once with 10 ml of phosphate-buffered saline (PBS, SIGMA). Cells

were removed from the bottom of the flask by incubating with 1.0 ml of trypsin (GIBCO) for five minutes. 1.0 ml of DMEM was added to the flask, cells were titrated with a pasteur pipette and collected into a 15 ml conical polypropylene tube (Falcon). 10 ml of DMEM was added to the tube and cells were spun-down on a table-top swing-arm centrifuge for 30 seconds. The medium was aspirated and replaced with 2 ml of DMEM. The resultant cell pellet was resuspended first using a 20-1/2 gauge needle followed by a 22-1/2 gauge needle. 8 ml of DMEM was added to make a final volume of 10 ml. 13  $\mu$ l of this cell suspension was taken for cell counting using a haemocytometer. A dilution was performed using DMEM to make a final cell suspension with a density of  $1.0 \times 10^6$  cells/ml. 10 mls of cells from each treatment group was pipetted into 15 ml conical tubes labeled CONTROL, NGF, or RU-486. Drugs were reintroduced to the cells at the same concentration as described above for the 12-hour pretreatment. NGF (50 ng/ml) was added to the tube of cells labeled 'NGF' as a positive control. 1.0 ml of cells from each group was pipetted into appropriately labeled 1.5 ml eppendorf tubes (BIORAD) and placed into a 5% CO<sub>2</sub> incubator equilibrated at 37°C for fifteen minutes.

After the fifteen minutes had elapsed, the tubes were placed immediately on ice to stop any reactions and 200  $\mu$ l of a protease cocktail (100  $\mu$ M PMSF (phenylmethanesulfonyl fluoride), 5  $\mu$ g/ml Leupeptin, 5  $\mu$ g/ml Aprotinin, and 5  $\mu$ g/ml Pepstatin) was added to each tube. Cells were pelleted at 14,000 rpm for five minutes at 4°C and the medium was removed and collected into fresh, ice-cold 1.5 ml eppendorf tubes containing protease inhibitor cocktail. Cell pellets were lysed using 50  $\mu$ l of met/lysis buffer (10 mM Tris-HCl, 150 mM NaCl, 1% Nonidet P40 (v/v), and 1% Na deoxycholate (w/v), pH 7.4). The nuclear fraction was pelleted down at 14,000 rpm for ten min. and 5  $\mu$ l of cellular homogenate was collected for a protein quantification.

APP<sub>S</sub>-containing medium was desalted using ultrafree-CL centrifugation filters with a 30,000 MW cut-off range membrane (Millipore). The desalted supernatant was placed in 1.5 ml eppendorf tubes and concentrated using a speed vac-concentrator. 30  $\mu$ l of Laemmli sample buffer (0.0625 Tris-HCL, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromophenol blue) was added to reconstitute the pellet.

A bicinchoninic acid (BCA) protein assay kit from Pierce (PO Box 117, Rockford, Illinois, 61105) was used to perform protein quantification on the cellular extracts. 5  $\mu$ l of each sample was taken for measurements, according to manufacturer's instructions.

Secreted protein corresponding to 10  $\mu$ g of cellular protein from each treatment group were boiled at 100°C for four minutes and separated by tris-glycine SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Protein bands were

transferred onto nitrocellulose membranes and Western blotting using the 22C11 monoclonal antibody (monoclonal antibody against APP) was performed (Mills, J. and P. B. Reiner, "Phorbol esters but not the cholinergic agonists oxotremorine-M and carbachol increase release of the amyloid precursor protein in cultured rat cortical neurons", *J. Neurochem.* 67:1511-1518 (1996). Bands were visualized using an enhanced chemiluminescence "ECL" kit from Amersham Canada Limited, Oakville, Ontario and detected on ECL Hyperfilm (Amersham). Protein bands were quantified by densitometry of the films. APP<sub>S</sub> usually ran as two or three close bands on the gel corresponding to the different isoforms of APP<sub>S</sub> and they were quantified together and denoted APP<sub>S</sub>.

RU-486 increased APP<sub>S</sub> in the GSrasDNI PC12 cells (Fig. 1). This cell line was used to examine the role of *ras* in regulating APP<sub>S</sub> secretion. RU-486 was used as a negative control group for the expression of the glucocorticoid-inducible MMTV promoter-linked dominant negative *ras* gene product in these cells. In these experiments, the effects of prolonged exposure of RU-486 (twelve hours) on these cells were studied and APP<sub>S</sub> was collected for fifteen minutes. NGF is a known enhancer of APP<sub>S</sub> secretion in PC12 cells (Fukuyama, R. *et al.*, "Nerve growth factor-induced neuronal differentiation is accompanied by differential induction and localization of the amyloid precursor protein (APP) in PC12 cells and variant PC12S cells", *Mol. Brain Res.* 17:17-22 (1993); Haring, R. *et al.*, "NGF promotes amyloid precursor protein secretion via muscarinic receptor activation", *Biochem. Biophys. Res. Comm.* 213:15-23 (1995), the teachings of which are hereby incorporated by reference) and was used as a positive control in this study. The increase in APP<sub>S</sub> secretion due to RU-486 was found to be ~12.5-fold increase over control samples and ~6 fold increase over NGF (Fig. 2).

The evidence demonstrates that blockade of p-glycoprotein increases APP<sub>S</sub> release from cells. It is art recognized that when release of APP<sub>S</sub> is increased that the release of A $\beta$  is decreased (Buxbaum *et al.*, Protein phosphorylation inhibits production of Alzheimer amyloid  $\beta$ /a4 oetudem *Proc. Natl. Acad. Sci.* 90:9195-9198 (1993); Fukushima *et al.*, Activation of the secretory pathway leads to a decrease in the intracellular amyloidogenic fragments generated from the amyloid protein precursor, *Biochem. Biophys. Res. Comm.* 194:202-207 (1993); Gabuzda *et al.*, Inhibition of  $\beta$ -amyloid production by activation of protein kinase C., *J. Neurochem.* 61:2326-2329 (1993); Hung *et al.*, Activation of protein kinase C inhibits cellular production of the amyloid  $\beta$ -protein, *J. Biol. Chem.* 268:22959-22962 (1993); Jacobsen *et al.*, The release of Alzheimer's disease  $\beta$ -amyloid peptide is reduced by phorbol treatment, *J. Biol. Chem.* 269:8376-8382 (1994); Wolf *et al.*, Muscarinic regulation of Alzheimer's disease

amyloid precursor protein secretion and amyloid  $\beta$ -protein production in human neuronal NT2N cells, *J. Biol. Chem.* 270:4916-4922 (1995)).

## II. Concentration Effects of RU-486 On APP<sub>s</sub> Generation

5       The materials and methods used herein are as described in Example 1. Various concentrations of RU-486 were used to treat GSrasDN1 PC12 cells. Cells were exposed to final concentrations of 3.0 to 3000.0 nM of RU-486. Drug exposure protocol was carried-out as previously described. Determination of the concentration and quantification of APP<sub>s</sub> was also performed as previously described.

10       Results indicated that increased APP<sub>s</sub> secretion in GSrasDN1 PC12 cells is RU-486 dose-dependent (Fig. 3), with a half-maximal effect at about 0.5  $\mu$ M (Fig. 4). Concentration effect studies above 3.0  $\mu$ M RU-486 became difficult because RU-486 did not remain a homogenous aqueous solution above that concentration. However, even at sub-maximal concentrations of RU-486, increased APP<sub>s</sub> secretion is noted (~21-  
15 fold at 3.0  $\mu$ M RU-486, Fig. 4).

## III. The Use of a Second P-glycoprotein Blocker

Phorbol 12-myristate 13-acetate (PMA) was purchased from LC Service Corp., Woburn, Mass. All other materials were obtained as previously described in Example 1.  
20 Wild-type PC12 cells were purchased from the American Type Tissue Culture Collection # CRL-1721 and grown in DMEM with 5% FBS fetal bovine serum and 10% horse serum (GIBCO).

PC12 cells grown to confluence were pretreated for twelve hours in DMEM supplemented with charcoal-stripped calf serum (SIGMA) as described above. After  
25 pretreatment, cells were trypsinized, resuspended, and counted as described above. 10 mls each of  $1.0 \times 10^6$  cells/ml PC12 cells were aliquoted into 15 ml conical tubes containing the appropriate treatment group. PMA was used as a positive control as previous experiments had shown that such treatment increases APP<sub>s</sub> release (Buxbaum, J. D. *et al.*, "Processing of Alzheimer  $\beta$ /A4 amyloid precursor protein: Modulation by  
30 agents that regulate protein phosphorylation", *Proc. Natl. Acad. Sci. USA* 87:6003-6006 (1990); Caporaso, G. L. *et al.*, "Protein phosphorylation regulates secretion of Alzheimer  $\beta$ /A4 amyloid precursor protein", *Proc. Natl. Acad. Sci. USA* 89:3055-3059 (1992); Mills, J. and P. B. Reiner, "Phorbol esters but not the cholinergic agonists oxotremorine-M and carbachol increase release of the amyloid precursor protein in cultured rat cortical  
35 neurons", *J. Neurochem.* 67:1511-1518 (1996), the teachings of which are hereby incorporated by reference). The final percentage of DMSO in all treatment groups was normalized to 0.01% (v/v).

Treatment groups were as follows: a) Control; b) PMA (0.1  $\mu$ M); c) RU-486 (0.1  $\mu$ M). The concentration of (0.1  $\mu$ M) RU-486 was chosen because it was near the EC<sub>50</sub> mark (Fig. 4). 1.0 ml of each treatment group was pipetted into 1.5 eppendorf tubes and incubated at 37°C in a 50% CO<sub>2</sub> incubator for fifteen minutes. Harvesting and  
5 concentration of APP<sub>s</sub> were performed as described in Example 1. Protein band separation on SDS-PAGE, visualization of bands, and quantification of APP<sub>s</sub> were also performed as previously described.

RU-486 increased APP<sub>s</sub> secretion in wild-type PC12 cells following fifteen minute exposure (Fig. 5). The data indicated that the effect previously shown was not  
10 due to an effect caused by the presence of the dominant-negative *ras* gene in the GSrasDN1 PC12 cell line. Since it is generally accepted that genomic effects of steroid hormone receptor activation require at least one hour to manifest (Wehling, M, *Trends Endocrinol Metab.* 56:347-353, 1994), the data demonstrate that a twelve hour pretreatment with RU-486 was not necessary for an RU-486-mediated increase in APP<sub>s</sub>  
15 secretion. The data also demonstrated that APP<sub>s</sub> secretion was not dependent upon a change in gene transcription.

Fig. 5 depicts a western blot which shows varying intensities in APP<sub>s</sub> isoforms, most likely due to variations in post-translational processing of isoforms. Since each band shows corresponding changes upon treatment with RU-486, it can be concluded  
20 that RU-486 affects the release of all isoforms of APP<sub>s</sub>, although subtle isoform selective effects have not been ruled out. The increase in APP<sub>s</sub> secretion with PMA confirms published findings (Buxbaum, J. D. *et al.*, "Processing of Alzheimer  $\beta$ /A4 amyloid precursor protein: Modulation by agents that regulate protein phosphorylation", *Proc. Natl. Acad. Sci. USA* 87:6003-6006 (1990); Caporaso, G. L. *et al.*, "Protein  
25 phosphorylation regulates secretion of Alzheimer  $\beta$ /A4 amyloid precursor protein", *Proc. Natl. Acad. Sci. USA* 89:3055-3059 (1992); Mills, J. and P. B. Reiner, "Phorbol esters but not the cholinergic agonists oxotremorine-M and carbachol increase release of the amyloid precursor protein in cultured rat cortical neurons", *J. Neurochem.* 67:1511-1518 (1996)) and give assurance that the cells used in the experiments are biochemically  
30 viable.

#### IV. Determining Whether RU-486 Acts Through Steroid Hormone Receptors to Increase APP<sub>s</sub> Secretion

E82 mouse L-cells were generously provided by Dr. Mark Danielson  
35 (Georgetown University, USA). All materials and methods are as described above.

E82 cells were grown in DMEM with 5% FBS and cultured in 75 cm<sup>2</sup> flasks from Falcon.

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Concentration of and quantification of APP<sub>S</sub> were also performed as described above.

RU-486 increases APP<sub>S</sub> release from E82 cells. These cells (Housley, P. R. and Forsthoefel, A. M., Isolation and characterization of a mouse L cell variant deficient in glucocorticoid receptors, *Biochem. Biophys. Res. Comm.* 164:480-487, 1989)) lack steroid hormone receptors with which RU-486 is known to interact, e.g., the glucocorticoid and progesterone receptors (Baulieu, *Science* 245:1351-1357, 1989). The increase in APP<sub>S</sub> secretion using RU-486 in these cells (Fig. 6) further demonstrates that the observed effects occurs independent of RU-486 activity at steroid hormone receptors.

In western blots probing for APP<sub>S</sub> secretion in E82 cells, only two isoform bands of APP<sub>S</sub> appeared (Fig. 6) compared to the three bands seen in those using PC12 cells (Figs. 1, 3, 5). This was attributed to the differences in maturation of APP<sub>S</sub> isoforms between cell lines.

#### V. Demonstration that Three Different P-glycoprotein Blockers Increase APP<sub>S</sub> Secretion in Wild Type PC12 Cells

Materials and methods utilized were as outlined above. Dexamethasone was purchased from Sigma. Progesterone (HBC complex) and desmethoxyverapamil were purchased from Research Biochemicals Inc.

Treatment groups were as follows: a) Control; b) PMA (0.1 μM); c) RU-486 (0.1 μM); d) dexamethasone (0.1 μM); progesterone (1 μM); desmethoxyverapamil (1 μM).

Fifteen minute exposure of PC12 cells to RU-486, progesterone and desmethoxyverapamil significantly increased APP<sub>S</sub> secretion from PC12 cells. (Fig. 7 and Fig. 8). Therefore, it was determined that these therapeutic drugs act as p-glycoprotein blockers. As a negative control, the steroid hormone receptor agonist dexamethasone, which is transported by p-glycoprotein but is not known as a p-glycoprotein blocker was included; it did not significantly increase APP<sub>S</sub> secretion. These data represent pharmacological evidence that blockade of p-glycoprotein alters APP catabolism.

#### VI. Effect of RU-486 on a Human Carcinoma Cell Line; No Increased Production of APP<sub>S</sub>

A KB-3.1 human carcinoma cell line, expressing low levels of the human MDR1 gene encoding P-glycoprotein gene product (Shen *et al.*, 1986, *Science*, 232: 643-5), was obtained as a gift from Dr. Ira Pastan (National Cancer Institute, USA). These cells

were grown in DMEM with 10% FBS. All materials and methods are as outlined as above.

KB-3.1 cells expressing sub-detectable levels of p-glycoprotein lack modulation of APP<sub>s</sub> by RU-486 (Fig. 9). This experiment directly addresses the involvement of p-glycoprotein in regulating APP catabolism. The observation that cells having low expression of p-glycoprotein do not regulate APP<sub>s</sub> secretion either by RU-486 or PMA (Fig. 9) suggests that the catabolism of APP may be linked in some fashion to the level of expression of p-glycoprotein. Western blots were performed on PC12, E82, and KB-3.1 cells probing for cellular levels of p-glycoprotein. Detectable levels of the protein were found in PC12 and E82 cells, but none in KB-3.1 cells (data not shown). Although this experiment is not conclusive nor definitive in implicating p-glycoprotein as the only molecule underlying RU-486 mediated APP<sub>s</sub> increase, it does provide direct support for this belief.

#### VII. Transfection of pHaMDR1 Construct into KB-3.1 Cells Induces APP<sub>s</sub> Release

KB-3.1 cells were grown as described above. The pHaMDR1 plasmid construct encoding human MDR1 was obtained as a gift from Dr. Ira Pastan (National Cancer Institute).

For calcium-phosphate transfections, cells were plated onto 10 cm polypropylene petri dishes (Falcon) at a density of  $2.0 \times 10^6$  cells/dish. To prepare the cDNA for transfection, 15 µg/plate of pHaMDR1 cDNA was mixed together with 1/10 volumes of 3M sodium Acetate, ~3x volume of 100% ethanol in a 1.5 ml eppendorf tube and spun in a desktop centrifuge for thirty seconds. The tube was then filled to the top with ethanol and spun at 15,000 rpm at 4°C for ten minutes. The ethanol was aspirated, leaving a tiny pellet of cDNA on the bottom. In a sterile fumehood, the cDNA was resuspended with 450 µl of 0.1 x tris-EDTA(TE) ethylene diamine tetraacetic acid, tris EDTA and 50 µl of 2.5 M CaCl<sub>2</sub>. 500 µL of 2x BES (50 mM N, N-bis[2-Hydroxyethyl]-2-aminoethanesulfonic acid, 280 mM NaCl, 1.5 mM Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, pH 6.96) was added to make a final volume of 1.0 ml. The entire mixture was allowed to sit for twenty minutes before adding the contents to the plate of cells. Plates were placed in a 3% CO<sub>2</sub> incubator for 8-10 hours. The transfection was stopped by aspirating the media, washing the cells twice with ~5 of media and replating the cells onto poly-D-lysine (SIGMA)-coated 10 cm petri dishes at a density of  $2.0 \times 10^6$  cells/dish. Cells were left to adhere and then the medium was replaced with DMEM supplemented with 10% charcoal-stripped calf serum prepared twelve hours prior to the drug exposure. To assay for transfection efficiency, the β-galactosidase (β-gal) gene



was also co-transfected into the cells.  $\beta$ -gal staining was performed to determine the percentage of cells within each dish which were successfully transfected with the cDNA.

Exposures were performed without resuspending the cells, in contrast to previous experiments described above. 10 ml solutions of DMEM containing either control  
5 (DMSO), PMA(0.1  $\mu$ M) or RU-486 (0.1  $\mu$ M) were prepared. DMSO concentrations in the medium were normalized to 0.01%(v/v). The supplemented medium was aspirated and 1 ml of the appropriate medium was added to the cells. Dishes were returned to the incubator for fifteen minutes and the medium harvested and processed as previously described. The plated cells were lysed using 100  $\mu$ l of met/lysis buffer and harvested  
10 with cell scrapers (Falcon). 5  $\mu$ l from each sample was collected for protein quantification. Protein determination and APP<sub>s</sub> quantification were performed as previously described.

Transient transfection of the human MDR1 gene into the KB-3 cell line restored regulation of APP<sub>s</sub> secretion by PMA and RU-486 (Fig 10). This experiment suggests  
15 that the presence of the MDR1 gene protein product known as p-glycoprotein confers modulation of APP<sub>s</sub> secretion upon cells. Other members of the ABC superfamily of transporters can serve a similar function, in a fashion analogous to the ability of human p-glycoprotein to replace the function of yeast ABC transporter STE-6 in exporting the mating pheromone a-factor (Raymond, M. *et al.*, "Functional Complementation of Yeast  
20 *ste-6* by a mammalian Multidrug resistance *mdr* gene," Science 256:232 (1992)). That a subdetectable endogenous amount of p-glycoprotein is not sufficient to allow regulated APP<sub>s</sub> secretion indicates that there must be a threshold titre of p-glycoprotein which must be present in any cell in order for this modulation to occur.  $\beta$ -gal staining of the transfected cells showed a relatively low percent-transfection efficiency (~10-20%),  
25 thus, it was assumed that only 10-20% of the cells per dish had been transfected with MDR1. That an overall restoration in the regulation of APP<sub>s</sub> secretion by PMA and RU-486 (Fig. 10) could be seen with such a low transfection efficiency indicated that the effect of p-glycoprotein is potent in affecting this modulated secretion.

Protein bands migrated as only one strong band in the Western blot (Fig. 10).  
30 This was evident in experiments in which cells were exposed to drugs while plated, compared to when they were in cell suspension (as previously performed). To control against any anomalous effects which could have been due to this modified protocol, the same drug experiments were performed in non-transfected KB-3.1 cells. It was found that there was no regulation of APP<sub>s</sub> secretion by RU-486 or PMA, further  
35 demonstrating that plated versus suspended cells did not alter the principal findings of enhanced APP<sub>s</sub> secretion by RU-486 and PMA.

### VIII. RU486 reduces A $\beta$ secretion in SW cells

Figure 11 demonstrates that RU486, a P-gp blocker reduces A $\beta$  secretion in fifteen minutes in a dose-dependent manner in K269sw cells transiently transfected with the human pHaMDR1 construct. K269sw cells were cultured in 20 mm Falcon dishes in DMEM containing 10% FBS, and 200 mg/mL geneticin (GIBCO) to about 50% confluence and transiently transfected with the human pHaMDR1 construct using the calcium-phosphate precipitation technique as described above. Cells were replated at a density of  $2.0 \times 10^6$  cells/dish 8 hours post-transfection on 10 cm petri dishes as described above. To assay for transfection efficiency, the  $\beta$ -gal gene was also co-transfected into the cells.  $\beta$ -gal staining was performed to determine the percentage of cells within each dish which were successfully transfected with the cDNA.

Exposures were performed without resuspending the cells. 10 mL of DMEM containing either control (DMSO), PMA (0.1  $\mu$ M), or RU486 (0.001  $\mu$ M, 0.01  $\mu$ M, 0.1  $\mu$ M, 1.0  $\mu$ M), were prepared. DMSO concentrations in the medium were normalized to 0.01% (v/v). The supplemented medium was aspirated and 1 mL of the appropriate medium was added to the cells. Dishes were returned to the incubator for fifteen minutes and the medium harvested and processed as previously described. The plated cells were lysed using 100  $\mu$ L of met/lysis buffer and harvested with cell scrapers (Falcon). 5  $\mu$ L from each sample was collected for protein quantification.

Total protein in the harvested media was precipitated using a trichloroacetic acid (TCA) precipitation. TCA corresponding to 1/10 volume of total media was added to each of the 1.5 mL Eppendorf tubes containing harvested media and incubated at -20°C for 15 minutes. Protein was immediately pelleted in the tubes with a 14,000 rpm spin at 4°C for 4 minutes. The supernatant was aspirated, leaving the pellet of precipitated protein. The pellet was washed once with 10% TCA and resuspended in 20  $\mu$ L of Laemmli buffer.

SDS-PAGE was used to resolve secreted protein on a 16.5% tris-tricine polyacrylamide gel. Protein bands were then transferred onto nitrocellulose membranes (Biorad). Western blotting was then performed using the W0-2 rat monoclonal antibody specific for human A $\beta$  (gift from Konrad Beyreuther). A $\beta$  was visualized using ECL and quantitation was performed as previously described.

### IX. RU49953 reduces A $\beta$ Secretion in SW cells

Figure 12 demonstrates that RU49953, a selective P-gp blocker, reduces A $\beta$  secretion in fifteen minutes in a dose-dependent manner in K269sw cells transiently transfected with the human pHaMDR1 construct. Culturing and transient transfection of

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K269sw cells are as previously described. Preparation of cells for the drug assays are also performed as previously described.

Exposures were performed without resuspending the cells. 10 mL of DMEM containing either control (DMSO), PMA (0.1  $\mu$ M), or RU49953 (0.01  $\mu$ M, 0.1  $\mu$ M, 1.0  $\mu$ M), were prepared. DMSO concentrations in the medium were normalized to 0.01% (v/v). The supplemented medium was aspirated and 1 mL of the appropriate medium was added to the cells. Dishes were returned to the incubator for fifteen minutes and the medium harvested and processed as previously described. The plated cells were lysed using 100  $\mu$ L of met/lysis buffer and harvested with cell scrapers (Falcon). 5  $\mu$ L from each sample was collected for protein quantification.

Total protein in the harvested media was precipitated using a trichloroacetic acid (TCA) precipitation. TCA corresponding to 1/10 volume of total media was added to each of the 1.5 mL Eppendorf tubes containing harvested media and incubated at -20°C for 15 minutes. Protein was immediately pelleted in the tubes with a 14,000 rpm spin at 4°C for 4 minutes. The supernatant was aspirated, leaving the pellet of precipitated protein. The pellet was washed once with 10% TCA and resuspended in 10  $\mu$ L of Laemmli buffer.

SDS-PAGE was used to resolve secreted protein on a 16.5% tris-tricine polyacrylamide gel. Protein bands were then transferred onto nitrocellulose membranes (Biorad). Western blotting was then performed using the W0-2 rat monoclonal antibody specific for human A $\beta$  (gift from Konrad Beyreuther). A $\beta$  was visualized using ECL and quantitation was performed as previously described.

#### X. Transient Transfection of Human MDR1 in K269sw Cells Increases Basal Secretion of $\beta$ -Amyloid

Figures 13 and 14 demonstrates that transient transfection of P-glycoprotein in K269sw cells increases basal secretion of  $\beta$ -amyloid. K269sw cells were grown as previously described. Protocols for transient transfection are also as described albeit the following modifications. Individual 20 mm plates of K269sw cells were transfected with the following: control (no calcium-phosphate precipitation); mock (calcium-phosphate precipitation without any plasmid);  $\beta$ -gal (transfection with the  $\beta$ -galactosidase gene); and MDR1 (transfection with the human MDR1 gene). Replating of cells post-transfection was performed as described above.

Basal levels of A $\beta$  secretion were assayed in the following way. Supplemented medium was replaced with 1 mL DMEM and dishes were returned to the incubator for one hour. Conditioned medium was harvested and secreted A $\beta$  was detected and quantified as previously described.

**Equivalents**

Those skilled in the art will know, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention  
5 described herein. These and all other equivalents are intended to be encompassed by the following claims.

**CLAIMS**

What is claimed is:

- 5 1. A method for modulating amyloid deposition in a subject, comprising administering to a subject an effective amount of at least one transporter blocker for an ATP binding cassette (ABC) transporter, such that modulation of amyloid deposition occurs.
- 10 2. The method of claim 1, wherein said ABC transporter blocker prevents or inhibits said amyloid deposition.
3. The method of claim 1, wherein said ABC transporter blocker modulates the cleavage of amyloid precursor protein (APP).
- 15 4. The method of claim 1, wherein said ABC transporter blocker modulates proteolytic processing of APP, such that production of amyloid- $\beta$  protein (A $\beta$ ) is decreased.
- 20 5. The method of claim 1, wherein said ABC transporter blocker modulates proteolytic processing of APP, such that production of soluble amyloid precursor protein (APP<sub>S</sub>) is increased.
6. The method of claim 1, wherein said ABC transporter blocker modulates said
- 25 ABC transporter's ability to export A $\beta$  from a cell.
7. The method of claim 6, wherein said ABC transporter blocker inhibits export of A $\beta$  from a cell.
- 30 8. The method of claim 6, wherein said ABC transporter blocker is a blocker of *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.
- 35 9. The method of claim 1, wherein said ABC transporter blocker can be one or more lipophilic agents.

10. The method of claim 1, wherein said ABC transporter blocker acts to antagonize transport through one or more ABC transporters expressed in the brain or the cerebral microvasculature.
- 5 11. The method of claim 1, wherein at least one ABC transporter blocker is selected from the group consisting of RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine..
- 10 12. The method of claim 1, wherein at least one ABC transporter blocker is selected from the group consisting of RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine..
- 15 20 13. The method of claim 1, wherein the ABC transporter blocker is not RU-486.
14. The method of claim 10, wherein at least one of said ABC transporters is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 25 352188 or 422562.
15. A method for treating a disease state associated with amyloidosis, comprising: administering to a subject an effective amount of at least one ATP binding cassette (ABC) transporter blocker, or a pharmaceutically acceptable salt thereof, such 30 that a disease state associated with amyloidosis is treated.
16. The method of claim 15, wherein said disease state is amyloid deposition associated with Alzheimer's Disease.
- 35 17. The method of claim 15, wherein said disease state is Alzheimer's Disease.

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18. The method of claim 15, wherein said ABC transporter blocker is a lipophilic agent.

19. The method of claim 15, wherein said ABC transporter is a *MDR1*, *MDR3*,  
5 *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.

20. The method of claim 15, wherein at least one ABC transporter blocker is selected  
10 from the group consisting of RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710,  
15 VX-853, GF120918, XR-9051 and trifluoperazine.

21. The method of claim 15, wherein at least one ABC transporter blocker is selected from the group consisting of RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788,  
20 AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.

25 22. The method of claim 15, wherein the ABC transporter blocker is not RU-486.

23. The method of claim 25, wherein at least one of said ABC transporters is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763,  
30 352188 or 422562.

24. A method for treating Alzheimer's disease, comprising administering to a subject having Alzheimer's disease an effective amount of at least one ATP binding cassette (ABC) transporter blocker, or a pharmaceutically acceptable salt thereof, such that  
35 treatment occurs.

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25. The method of claim 24, wherein said ABC transporter blocker prevents or inhibits Alzheimer's disease.
26. The method of claim 24, wherein said ABC transporter is *MDR1*, *MDR3*, *ABC1*,  
5 *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.
27. The method of claim 24, wherein said ABC transporter blocker can be one or more lipophilic agents.
- 10 28. The method of claim 24, wherein said ABC transporter blocker acts to antagonize transport of A $\beta$  through one or more ABC transporters expressed in the brain or the cerebral microvasculature.
- 15 29. The method of claim 24, wherein at least one ABC transporter blocker is selected from the group consisting of RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine,  
20 propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.
30. The method of claim 24, wherein at least one ABC transporter blocker is selected from the group consisting of RU-49953, verapamil, desmethoxyverapamil, cyclosporin,  
25 chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.
- 30 31. The method of claim 24, wherein the ABC transporter blocker is not RU-486.
32. The method of claim 24, wherein at least one of said ABC transporters is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC  
35 transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.



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33. A packaged pharmaceutical composition for treating amyloidosis, comprising:  
a container holding an effective amount of a pharmaceutical composition for  
modulating amyloid deposition in a subject, said pharmaceutical composition  
comprising at least one ATP binding cassette (ABC) transporter blocker and  
5 instructions for using said pharmaceutical composition for treatment of  
amyloidosis.
34. The packaged pharmaceutical of claim 33, wherein the pharmaceutical  
composition includes at least one lipophilic agent.
- 10 35. The packaged pharmaceutical of claim 34, wherein at least one ABC transporter  
blocker is selected from the group consisting of RU-486, RU-49953, verapamil,  
desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine,  
FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin,  
15 yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange,  
quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-  
dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.
36. The packaged pharmaceutical of claim 34, wherein at least one ABC transporter  
20 blocker is selected from the group consisting of RU-49953, verapamil,  
desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine,  
FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin,  
yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange,  
quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-  
25 dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.
37. The packaged pharmaceutical of claim 34, wherein the ABC transporter blocker  
is not RU-486.
- 30 38. The packaged pharmaceutical of claim 34, wherein the pharmaceutical  
composition is effective as a blocker of *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*,  
*ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597,  
122234, 123147, 131042, 157481, 182763, 352188 or 422562.
- 35 39. A method for identifying agents which modulate amyloid deposition in an  
organism, comprising administering to an organism an effective amount of at least one

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ATP binding cassette (ABC) transporter blocker, such that modulation of amyloid deposition occurs.

40. The method of claim 39, wherein said ABC transporter blocker modulates said  
5 ABC transporter's ability to export A $\beta$  from a cell.

41. The method of claim 40, wherein said ABC transporter blocker inhibits export of A $\beta$  from a cell.

10 42. The method of claim 40, wherein said ABC transporter is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.

43. A method for identifying agents which modulate transport of A $\beta$  across a cellular  
15 membrane, comprising introducing an agent into a model system containing a cellular membrane, an ABC transporter and A $\beta$  and measuring the ability of the agent to modulate the transport of A $\beta$  across the cellular membrane.

44. The method of claim 43, wherein the model system is an inside out plasma  
20 vesicle, an ABC transporter and A $\beta$ .

45. The method of claim 43, wherein said model system is a planar lipid bilayer, an ABC transporter and A $\beta$

25 46. A method for treating stroke, comprising administering to a subject having or having had a stroke an effective amount of at least one ATP binding cassette (ABC) transporter blocker, or a pharmaceutically acceptable salt thereof, such that treatment of said stroke occurs.

30 47. The method of claim 46, wherein said ABC transporter blocker modulates the cleavage of amyloid precursor protein (APP).

48. The method of claim 46, wherein said ABC transporter is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by  
35 the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.

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49. The method of claim 46, wherein at least one ABC transporter blocker is selected from the group consisting of RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.
50. A method for treating head injury, comprising administering to a subject afflicted with a head injury an effective amount of at least one ATP binding cassette (ABC) transporter blocker, or a pharmaceutically acceptable salt thereof, such that treatment of said head injury occurs.
51. The method of claim 50, wherein said ABC transporter blocker modulates proteolytic processing of APP, such that production of amyloid- $\beta$  protein (A $\beta$ ) is decreased.
52. The method of claim 50, wherein at least one ABC transporter blocker is selected from the group consisting of RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.
53. A method for modulating amyloid deposition in a subject, comprising administering to a subject an effective amount of at least one blocker of flippase activity for an ATP binding cassette (ABC) transporter, such that modulation of amyloid deposition occurs.
54. The method of claim 53, wherein said flippase blocker prevents or inhibits said amyloid deposition.
55. The method of claim 53, wherein said flippase blocker modulates the cleavage of amyloid precursor protein (APP).

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56. The method of claim 53, wherein said flippase blocker modulates proteolytic processing of APP, such that production of amyloid- $\beta$  protein (A $\beta$ ) is decreased.

57. The method of claim 53, wherein said flippase blocker modulates proteolytic processing of APP, such that production of soluble amyloid precursor protein (APP<sub>s</sub>) is increased.

58. The method of claim 53, wherein said flippase blocker modulates said ABC transporter's ability to export A $\beta$  from a cell.

10

59. The method of claim 58, wherein said flippase blocker inhibits export of A $\beta$  from a cell.

60. The method of claim 53, wherein said ABC transporter is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.

15

61. The method of claim 53, wherein said flippase blocker can be one or more lipophilic agents.

20

62. The method of claim 53, wherein said flippase blocker acts to antagonize transport of A $\beta$  through one or more ABC transporters expressed in the brain or the cerebral microvasculature.

63. The method of claim 53, wherein at least one flippase blocker is selected from the group consisting of RU-486, RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol, atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.

25

30

64. The method of claim 53, wherein at least one flippase blocker is selected from the group consisting of RU-49953, verapamil, desmethoxyverapamil, cyclosporin, chloroquine, quinine, chinchonidine, primaquine, FK-506, MS-209, MS-073, S 9788, AHC-52, tamoxifen, dihydrocyclosporin, yohimbine, corynanthine, reserpine, physostigmine, acridine, acridine orange, quinacrine, chlorpromazine, propanolol,

35

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atropine, tryptamine, forskolin, 1, 9-dideoxyforskolin, PSC-833, VX-710, VX-853, GF120918, XR-9051 and trifluoperazine.

65. The method of claim 53, wherein said flippase blocker is not RU-486.

5

66. The method of claim 53, wherein at least one of said ABC transporters is *MDR1*, *MDR3*, *ABC1*, *ABC2*, *ABC3*, *ABC7*, *ABC8*, *MRP4*, *MRP5* or the human ABC transporters encoded by the ESTs 45597, 122234, 123147, 131042, 157481, 182763, 352188 or 422562.

10

67. A method for treating a disease state associated with amyloidosis, comprising: administering to a subject an effective amount of at least one flippase blocker, or a pharmaceutically acceptable salt thereof, such that a disease state associated with amyloidosis is treated.

15

68. A method for treating Alzheimer's disease, comprising administering to a subject having Alzheimer's disease an effective amount of at least one flippase blocker, or a pharmaceutically acceptable salt thereof, such that treatment occurs.

20

69. A method for treating head injury, comprising administering to a subject afflicted with a head injury an effective amount of at least one flippase blocker, or a pharmaceutically acceptable salt thereof, such that treatment of said head injury occurs.

25

70. A packaged pharmaceutical composition for treating amyloidosis, comprising: a container holding an effective amount of a pharmaceutical composition for modulating amyloid deposition in a subject, said pharmaceutical composition comprising at least one flippase blocker and instructions for using said pharmaceutical composition for treatment of amyloidosis.

30

71. A method for identifying agents which modulate flipping of amyloid across a cellular membrane, comprising introducing an agent into a model system containing a cellular or synthetic membrane, an ABC transporter and amyloid and measuring the ability of the agent to modulate the flipping of amyloid across the cellular membrane.

35

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**RU486 increases APP<sub>s</sub> release  
from PC12 cells**

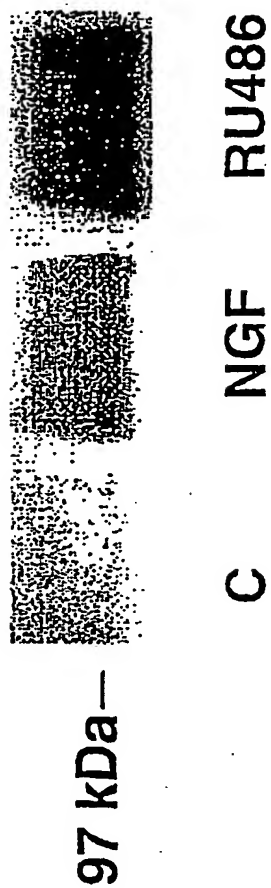


FIGURE 1

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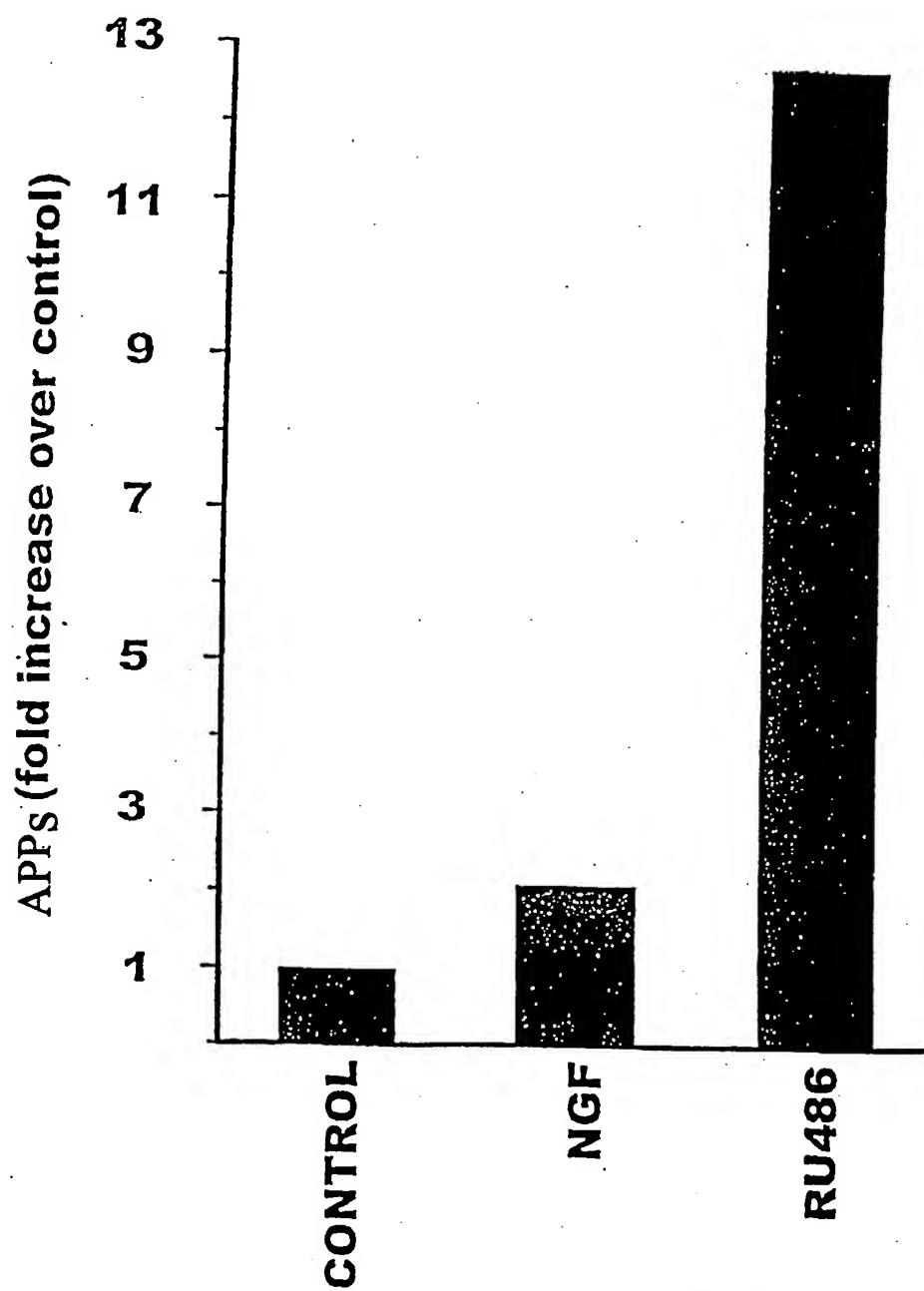


FIGURE 2

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**RU486 increases APP<sub>s</sub> release in a  
dose-dependent manner**

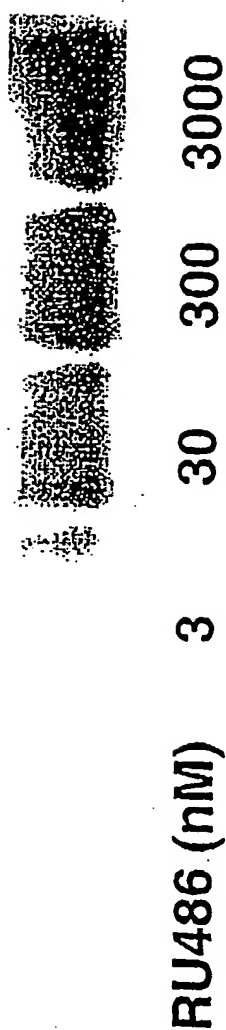


FIGURE 3



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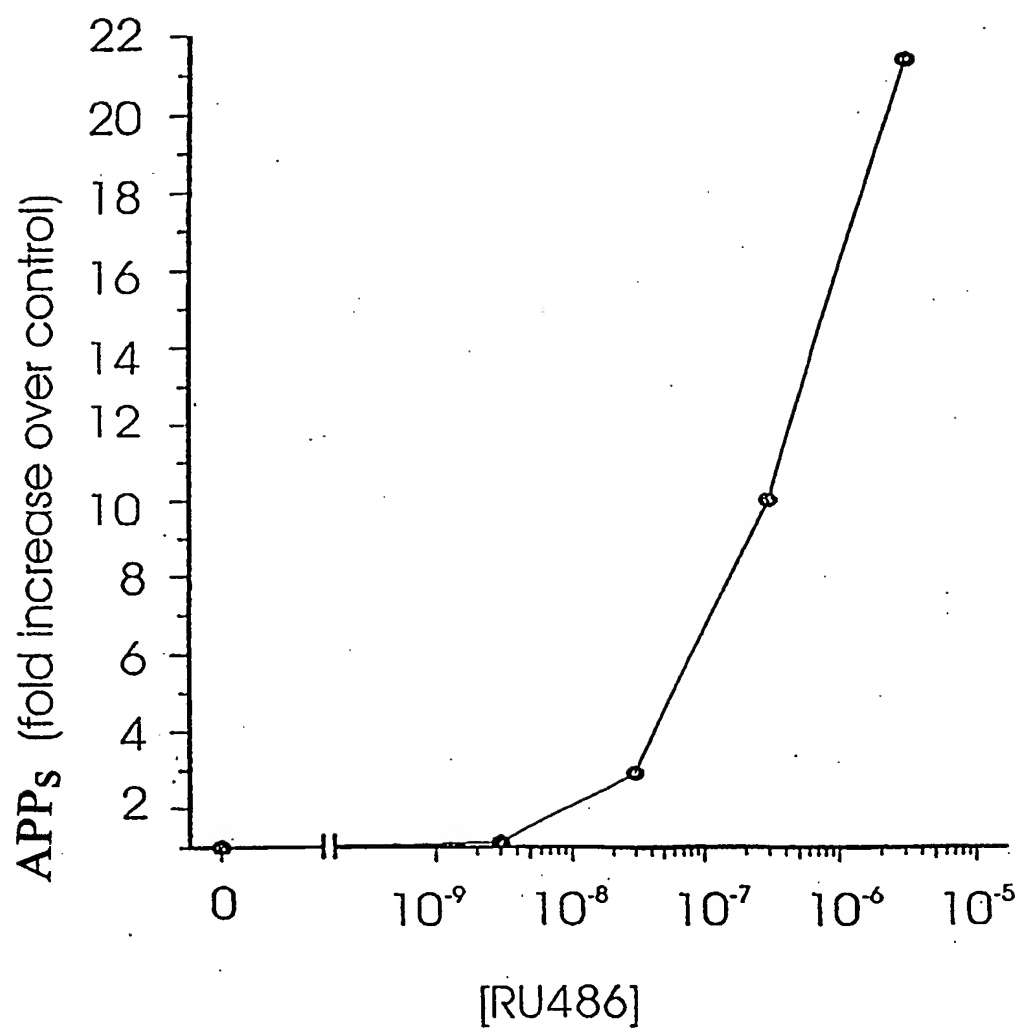


FIGURE 4

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**RU486 increases release of  
APP<sub>s</sub> in 15 minutes**

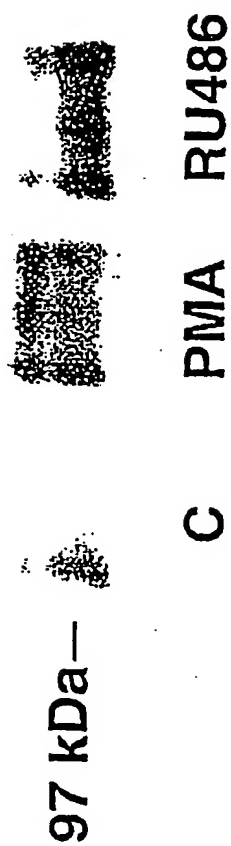


FIGURE 5

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# RU486 increases release of APP<sub>s</sub> in cells lacking GR and PR

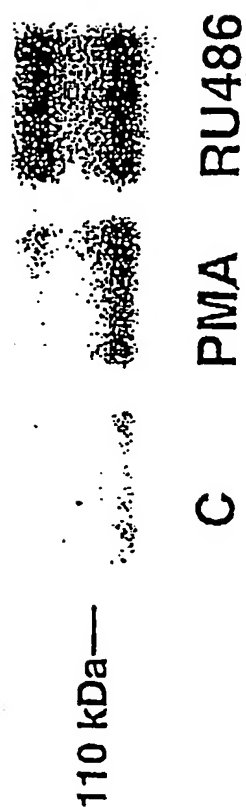


FIGURE 6

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RU486, Progesterone, and Desmethoxyverapamil Increase  
APPs Secretion in PC12 Cells in Fifteen Minutes

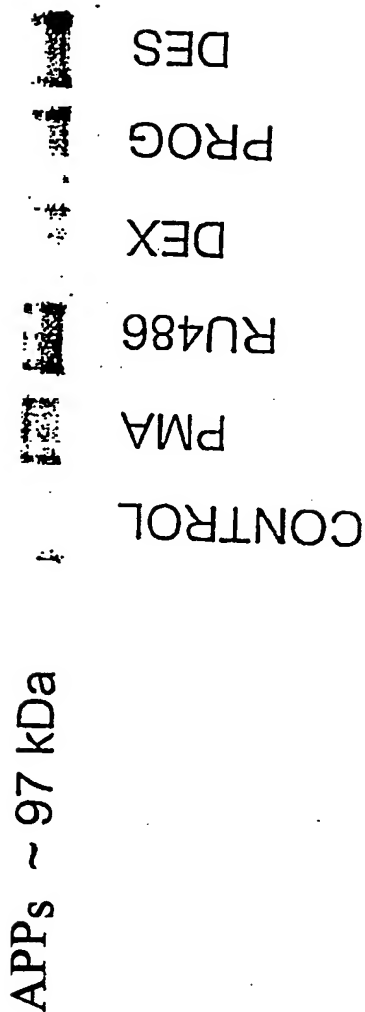


FIGURE 7

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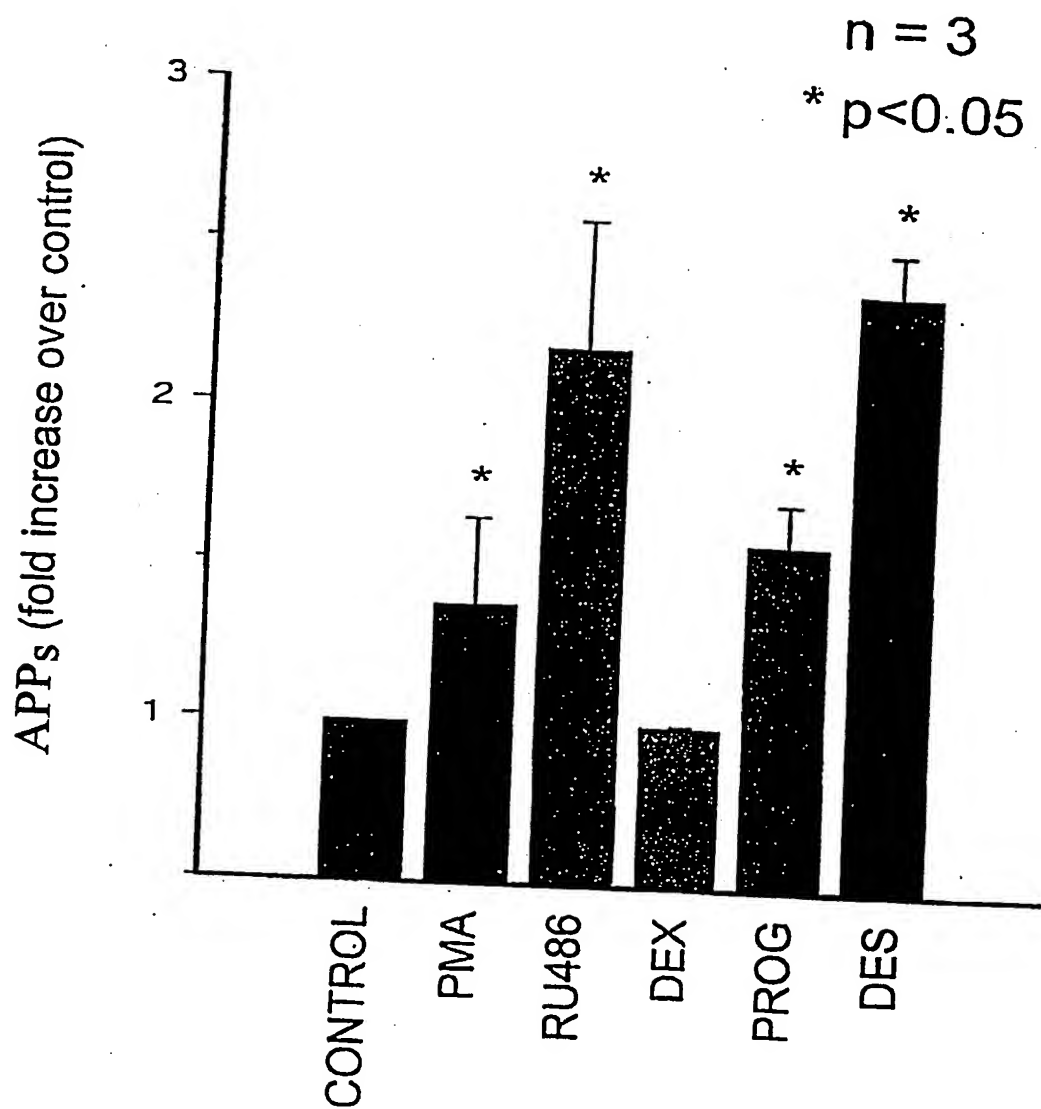


FIGURE 8

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RU486 does not regulate APP<sub>s</sub>  
secretion in cells which lack P-gp



FIGURE 9

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Transfection of KB3.1 cells with  
human P-gp restores regulated  
APP<sub>s</sub> secretion

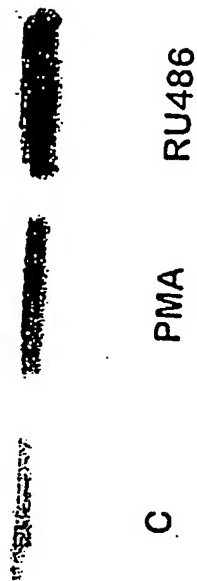


FIGURE 10

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RU486 Decreases A $\beta$  Secretion in SW Cells  
Transiently Transfected with Human MDR1

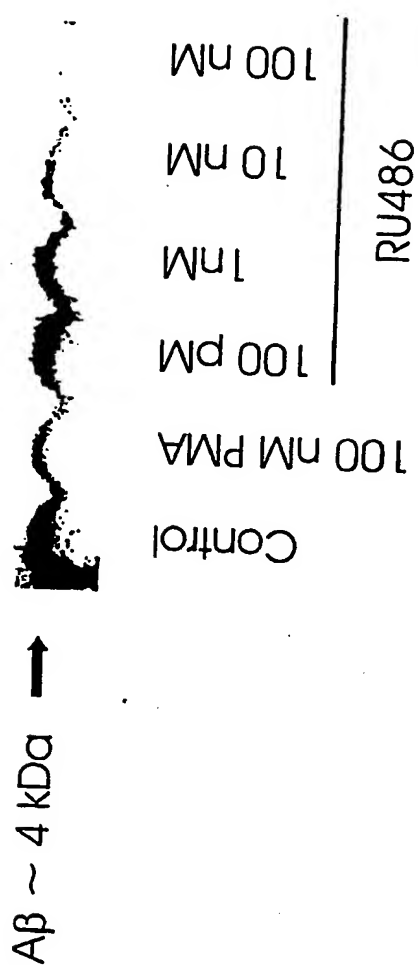


FIGURE 11



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RU49953 Decreases A $\beta$  Secretion in SW Cells  
Transiently Transfected with Human MDR1

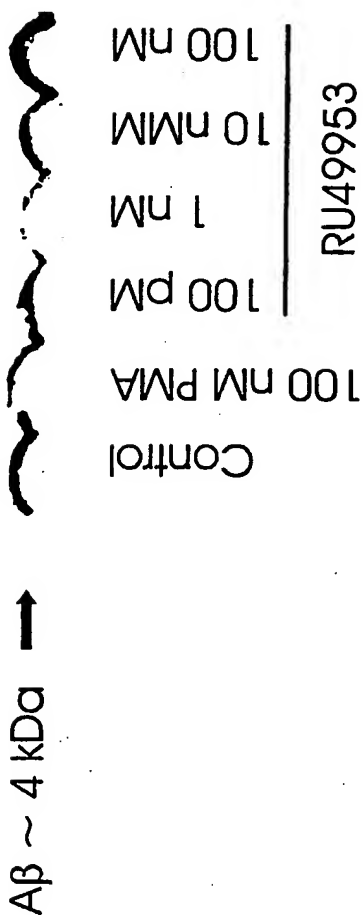


FIGURE 12

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Transient Transfection of P-glycoprotein Increases  
Basal Secretion of Beta-amyloid

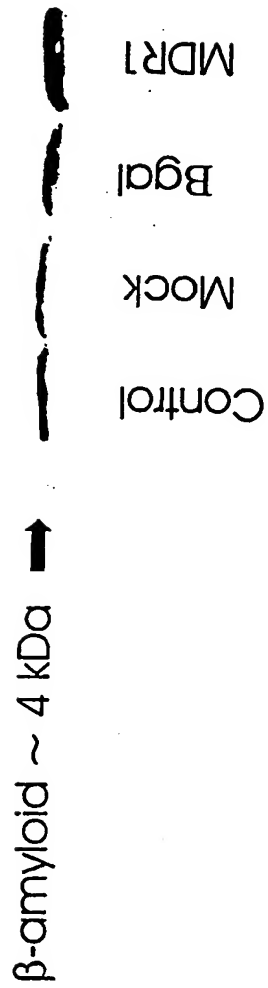


FIGURE 13

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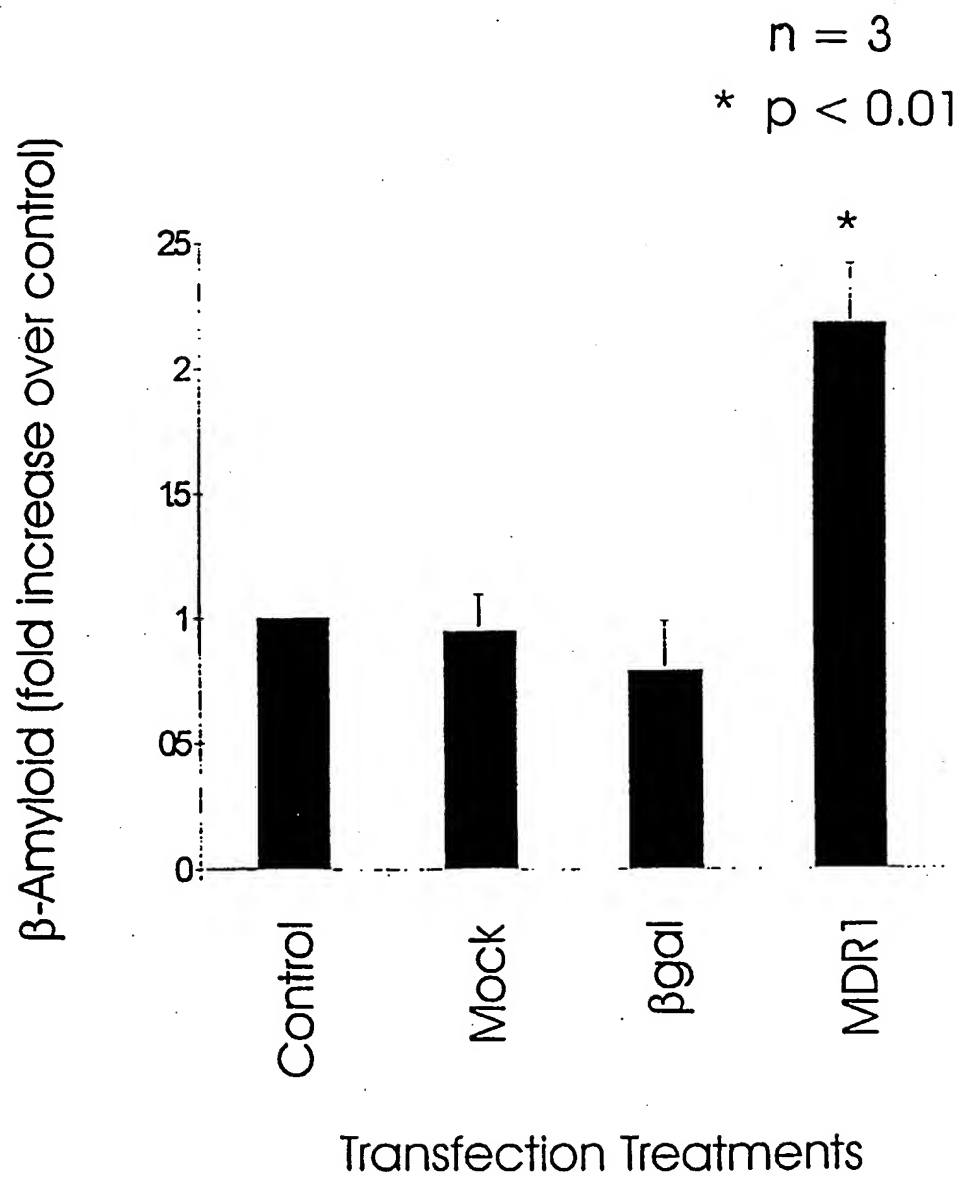


FIGURE 14

